New insights into the molecular effects and probiotic properties of *Lactobacillus pentosus* pre-adapted to edible oils

Esther Alonso García[1], Beatriz Pérez Montoro[1], Nabil Benomar[1], Sonia Castillo-Gutiérrez[2], María D. Estudillo-Martínez[2], Charles W. Knapp[3], Hikmate Abriouel[1]

[1]Área de Microbiología, Departamento de Ciencias de la Salud, Facultad de Ciencias Experimentales, Universidad de Jaén, 23071-Jaén, Spain.

[2]Área de Estadística e Investigación Operativa, Departamento de Estadística e Investigación Operativa, Facultad de Ciencias Experimentales, Universidad de Jaén, Jaén, Spain

[3]Centre for Water, Environment, Sustainability, and Public Health; Department of Civil and Environmental Engineering, University of Strathclyde, Glasgow, Scotland, United Kingdom.

[*]**Correspondence:** Dr. Hikmate Abriouel, Área de Microbiología. Departamento de Ciencias de la Salud. Facultad de Ciencias Experimentales. Edif. B3. Universidad de Jaén. Campus Las Lagunillas s/n. 23071-Jaén, Spain. Tel.: 34-953-212003; fax: 34-953-212943.
hikmate@ujaen.es

Abstract

In this study, the survival and growth of seven probiotic *Lactobacillus pentosus* strains isolated from Aloreña green table olives in the presence of vegetable-based edible oils (i.e., sunflower, olive, linseed, soy, corn, almond and argan) and mint essential oil were determined for the first time. Slight decreases in bacterial viability were observed depending on the strain and oil expsoure, mainly mint essential oil. However, pre-adapting the strains to the corresponding oils significantly increased their cell viabilities. As such, this study examined whether pre-adapting probiotic *L. pentosus* strains with oils will constitute a new strategy to increase stress resistance, e.g., acids (pH 1.5) or bile (up to 3.6%) in food production and/or during digestion, and improve functional probiotic properties. Improvements in stress resistance were noticed in some pre-adapted strains with oils, such as under acidic and bile conditions; further, pre-adaptations with olive, argan, sunflower and linseed oils induced gene expression (e.g., *fus*, *rpsL, pgm, groEL, enol* and *prep*) for moonlighting proteins involved in several stress responses and other functions. As such, pre-adaptation with vegetable edible oils may represent a novel approach for manufacturing probiotic products by improving the stability of bacteria during industrial processes that would otherwise reduce the viability and functionality of the strains.

Keywords:

Lactobacillus pentosus; Probiotics; Vegetable edible oils; Sub-lethal stress; qRT-PCR.

1. Introduction

Probiotics of vegetable origin have been increasingly gaining interest in the last ten years due to the demand for alternatively sourced probiotics from vegetarians and individuals with lactose intolerance, allergies, and dyslipidemia (Granato, et al. 2010), and by food manufacturers seeking different probiotics than those isolated from conventional sources (e.g., dairy products, human feces and breast milk). Lactic acid bacteria (LAB) represent a major group of probiotic bacteria including *Lactobacillus* spp. and *Bifidobacterium* spp., the most commonly used probiotics besides yeasts (Nousiainen, et al., 2004; Saulnier, et al., 2009). The autochtonous LAB isolated from vegetables have the capacity to survive under extreme environmental conditions such as acids, fluctuations of physical and nutritional conditions, high concentration of indigestible nutrients and anti-nutritional factors (Buckenhüskes, 1997; Rossi et al., 2005). In particular, the versatile species *L. pentosus* and *L. plantarum* have been found in a variety of environmental niches including naturally-fermented olives, which have been carriers of beneficial probiotic microorganisms capable to improve microbial balance in gastrointestinal tracts (Abriouel et al., 2012; Argyri, et al., 2016; Bautista-Gallego et al., 2013; Pérez Montoro et al., 2016).

Probiotics are defined by FAO/WHO (2002) as live microorganisms that, when administered in adequate amounts, confer a health benefit to the host. Thus, to biologically function, probiotics must remain viable during the processing, storage and transmission through the gastrointestinal tract (da Silva, de Fátima Bezerra, dos Santos, & Correia, 2015). Taking into account that viability is the most important parameter, there have been several strategies and approaches aimed to improve their survivability, such as immobilization in edible films or enclosed matrix (Ebrahimi et al., 2018; Nualkaekul, et al., 2013). However, other methods could be used, including stress-adaptation of probiotic bacteria, which may trigger the induction of proteins known to improve their survivability and resistance to forthcoming environmental, technological and gastrointestinal conditions (Casado Muñoz et al., 2016; De Angelis & Gobbetti, 2011, Pérez Montoro et al., 2018).

On the other hand, questions usually arise regarding the effect of diet or probiotics on the microbial diversity of the gut; however, the effect of diet on probiotic functionality should also be considered. As such, probiotics should include the exogenous bacteria administered and also those autochthonous, or indigenous, of the gut. Overall, diets can contain several susbstances that can enhance the activity of probiotics, such as prebiotics, and compounds that can inhibit or decrease (i.e., stress) the probiotic activity of some strains (Markowiak & Śliżewska, 2017; Ranadheera, et al., 2010). Treatments have been strategically sought to improve the stability of probiotics in terms of survivability and activity, since probiotics are fastidious and nutritionally exigent and sensitive to environmental conditions. Thus, dietary components such as edible oils could play an important role to change probiotic activities. In this sense, several reports described the use of some edible oils (e.g., fish oil, olive oil, rice-bran oil, and soybean oil) in prebiotic formulations that provided long-term protection to the organism and help maintain their proven probiotic properties and increased life span and shelf life (Baksh, 2014). Vegetable edible oils such as olive oil, sunflower oil, lineseed, soy, corn, almond and argan are common in several diets depending on the geographical region; however, there remains a knowledge gap on their effect on probiotics of vegetable origin such as *Lactobacillus* sp.

Several reports describe the responses of lactobacilli to stresses such as extreme temperature, pH, osmotic pressure, oxygen, and starvation, which physiologically affects the cells. Their physiological and molecular mechanisms involved in stress response include the induction of a specific proteins leading to possible increases in specific (i.e., a targeted response) or multiple stress (generic response) tolerances. Among the overexpressed proteins in lactobacilli include DnaK, GroEL, 30S-ribosomal proteins S1 and S6, ATP synthase subunit beta, MetK, phosphopyruvate hydratase, phosphoglycerate kinase, elongation factor Tu, putative manganese-dependent inorganic pyrophosphatase, D-lactate dehydrogenase, triosephosphate isomerase, fructosebisphosphate aldolase, and nucleoside-diphosphate kinase, related to quorum sensing (QS) and stress response mechanisms have been induced following exposure to several stressors (De Angelis & Gobbetti, 2011). Here, new insights into the molecular responses of *L. pentosus* pre-adapted with vegetable edible oils were also provided and attempt to determine whether edible oil adaptation influence their probiotic activities.

Specifically, this study assessed (for the first time) the effect of vegetable edible oils on seven probiotic *L. pentosus* strains isolated from naturally-fermented Aloreña green table olives (Abriouel et al., 2012; Pérez Montoro et al., 2016) with the aim to use the prebiotic oils in microencapsulation-based formulations. Furthermore, the possibility whether one could pre-adapt probiotics with oils to enhance their probiotic activities, such as tolerance to acids and bile salts and thus improve their stability in food production and effectiveness within the intestinal tract, was investigated.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Seven *Lactobacillus pentosus* strains, isolated from naturally-fermented Aloreña green table olives (Abriouel et al., 2012) with probiotic potentials (Pérez Montoro et al., 2016), were used in this study. These strains were routinely cultured at 37ºC in de Man Rogosa and Sharpe (MRS) broth (Fluka, Madrid, Spain) or agar under aerobic conditions for 24-48 h. Cultures were maintained in 20% glycerol at -20°C and -80°C for short- and long-term storage, respectively.

2.2. The effect of oils on survival and growth of L. pentosus strains

To determine the effect of different vegetable edible oils (i.e., sunflower, olive, linseed, soy, corn, almond and argan) and the essential mint oil on survival and growth of *L. pentosus* strains, an overnight culture of each strain grown in MRS broth at 37ºC was inoculated at 2% v/v in fresh MRS broth added with 2% v/v of each oil. Growth monitoring was done in a 96-well plate (200 µl per well) by measuring the optical density at 600 nm each hour for 23 h while incubating at 37ºC. To verify, serial dilutions of the samples were plated onto MRS agar plates at different time intervals (0, 8 and 24 h) to determine viable bacterial counts (log_{10} CFU/ml) following incubation at 37°C for 48 h. Furthermore, pH monitoring was conducted in all treatments after 24 h of growth. Each experiment was done in triplicate.

2.3. Acid and bile tolerance in oil-adapted Lactobacillus pentosus strains

Overnight culture of each strain was inoculated (2% v/v) into fresh MRS broth and with different oils added at 2% v/v. Samples were incubated under aerobic conditions for 24 h at 37ºC, and they were then re-cultured three times in the same concentration of oil. On the fourth day, adapted-strains were re-cultured into fresh MRS broth without any oils and then kept in 20% glycerol at -20°C and -80°C for short- and long-term storage, respectively.

To compare the effect of adaptation on growth and survival in the presence of different oils, overnight cultures of each adapted *L. pentosus* strain from each oil was cultured in MRS broth with and without different oils added. Growth and survival rates of each adapted strains were measured.

Assays to determine whether oil adaptation had an effect on survivability under gastric conditions, including acidity (pH 1.5–2) and bile salts (1.8 and 3.6%). were done according to methods described by Millette et al. (2008). Simulated gastric fluid (SGF) was formulated (U.S. Pharmacopeia): 3.2 g/liter of pepsin (Sigma), 2.0 g/liter of NaCl, and pH adjusted to 1.5 or 2.0 by the addition of HCl (10 N). Volumes (0.5 ml) of overnight cultures in MRS broth were added to 9.5 ml of SGF and then incubated at 37°C under mild agitation (200 rpm) in a G24 environmental incubator shaker (New Brunswick Scientific Co. Inc., NJ). After 30 min of incubation, 10 ml of culture were harvested, centrifuged and resuspended in 1 ml of sterile phosphate-buffered saline (PBS; pH 7.4). Immediately, culture suspensions were serially diluted in 0.85% NaCl solution and plated onto MRS agar. Plates were incubated under aerobic conditions at 37°C for 48 h, and they were then examined visually for bacterial growth. As a control, PBS was used instead of SGF to determine the initial CFU/ml for each strain.

Regarding bile-salt tolerance, MRS broths amended with 0%, 1.8% or 3.6% w/v bile-salt mixture (Sigma B-3426) were inoculated with 2% v/v overnight cultures, and the growth and survival rates were then obtained by measuring the absorbance at 600 nm for 23 h in parallel with viable counts at different time intervals (0, 8 and 24 h) onto MRS agar. Plates were incubated at 37ºC for 24-48 h for log_{10} CFU/ml determinations.

2.4. Stress/tolerance genes in oil-adapted L. pentosus strains

2.4.1. Detection of stress/tolerance genes

Total genomic DNA was isolated from *L. pentosus* strains using DNA Extraction Kit (Xtrem Biotech SL, Granada, Spain) according to the manufacturer's instructions. DNAs were frozen at -20ºC until required. The detection of selected genes (*gapd*, coding for glyceraldehyde-3-phosphate dehydrogenase; *tuf*, coding for elongation factor Tu; *fus*, coding for elongation factor G; *prep*, coding for prepilin; *groEL*, coding for heat shock protein GroE; *enol*, coding for enolase; *adhes*, coding for adhesin; *pgm*, coding for phosphoglycerate mutase; and *rpsL*, coding for 30S ribosomal subunit protein S12) was done by PCR using primers designed in this study based on *L. pentosus* MP-10 genome sequence (Abriouel et al., 2016). Primers and annealing temperatures are described in Table 1.

2.4.2. Quantitative reverse-transcriptase PCR of stress/tolerance genes

RNA extractions were done using Direct-zol™ RNA Miniprep (Zymo Research, California, USA) according to the manufacturer´s instructions. RNA quantification and quality assessment were carried out using a NanoDrop 2000 spectrophotometer (Thermo Scientific). RNAs were adjusted to a concentration of 500 ng/ml and frozen at -80 ºC until required for analysis.

The expression of selected genes (Table 1) was analysed by quantitative, real-time PCR (qRT-PCR) using SensiFAST^{TM} SYBR & Fluorescein One-Step Kit (BIOLINE). Phenylalanyl-tRNA synthase alpha-subunit (*pheS*) gene was used as a housekeeping gene (Naser et al., 2005), and a no-template control (NTC) was used as a negative control. Quantitative PCRs (qPCRs) were performed in triplicate on a CFX96 TouchTM Real-Time PCR Detection System from BioRad using 2 Power SYBR green chemistry.

2.5. Statistical analysis

All analyses were done in triplicate. Statistical analyses of data were accomplished using Excel 2007 program to determine the average data ± standard deviations. Statistical treatment of pH data was conducted by analysis of variances (ANOVA) in Statgraphics Centurion XVI, software using Shapiro–Wilk test and the Levene test to check data normality; the two-sided Tukey's Test determined the significance of differences among strain or oil treatments, where a *P*-value of <0.05 was considered statistically significant.

3. Results

3.1. Evaluation of the protective/inhibitory effect of vegetable oils on L. pentosus strains

The influence of vegetable edible oils and mint (an essential oil) on the growth of potentially probiotic *L. pentosus* strains in MRS broth medium was examined. Based on the growth kinetics and survivalability, each *L. pentosus* strain responded differently to oils. Overall, the essential mint oil inhibited all *L. pentosus* strains by decreasing bacterial counts ≤1.2 log_{10} units after 24 h incubation at 37ºC, while almond, linseed or sunflower oils had antimicrobial effect against some *L. pentosus* strains (Fig. 1).

pH were recorded as a possible indicator of the impact from vegetable oils (edible and essential) on growing *L. pentosus*. After 24 h growth at 37ºC, individual *L. pentosus* strains did not exhibit any pH differences with each oil (pH 3.6-4.0) except mint essential oil, which resulted in pH 5.1-5.5 values (Fig. 2A). However, significant differences were detected between the seven *L. pentosus* strains representing 5 dissimilar groups, with strains *L. pentosus* MP-10/*L. pentosus* CF2-12 and *L pentosus* AP2-15/*L. pentosus* AP2-16 (each pair) belonging to the same homologous groups (Fig. 2B).

3.2. Influence of oil-adaptation on survival and growth of L. pentosus strains

Oil-adapted *L. pentosus* strains exhibited an improvement in their survival and growth kinetics (Fig. S1). In fact, oils such as mint or almond, which previously decreased the growth of *L. pentosus* strains, did not exhibit any inhibitory effect on pre-adapted cells. All bacteria experienced improved growth capacity throughout their incubation period (23 h), as observed by monitoring their absorbance at 600 nm and bacterial enumeration (Fig. S1). When the survival and growth of each adapted strain (with each oil) were evaluated against the eight oil treatments used in the present study, it was observed that pre-adaptation with some oils improved growth of *L. pentosus* strains in the presence of the same oil and others (Table 2);

this highly depended on the *L. pentosus* strain and the oil used. However, overall soy, olive, corn and argan induced growth improvements for most *L. pentosus* strains (Table 2).

3.3. Evaluation of probiotic features in oil-adapted L. pentosus strains

Clear differences in acid tolerance were observed among oil-adapted *L. pentosus* strains, depending on the pH tested and the oil used for adaptation. At pH 2.0 all bacteria similarly grew (whether pre-adapted or not with oil) regardless of the oil used for adaptation, and similarly as those in PBS (Table 3). However, treatment-related statistically significant differences were detected at pH 1.5, at which *L. pentosus* strains adapted with corn and argan (5/7 strains), and soy (4/7 strains) exhibited increased growth capacity than the non-adapted cells by 0.31-6.45 log_{10} units after 24-h incubation at 37ºC. However, other oils such as olive, almonds, sunflower and linseed provided protection against acidity for some *L. pentosus* strains (3/7 strains), increasing their counts by 0.26-5.2 log_{10} units after 24-h incubation at 37ºC (Table 3).

With regards to bile salts, olive, lineseed and argan oils increased bile tolerance (statistically significant with *P*-value of <0.05) of some *L. pentosus* strains at both concentrations (1.8 and 3.6%), increasing bacterial counts by 0.16-0.76 log_{10} units after 8/24-h incubation at 37ºC, followed by other oils such as sunflower, almonds or corn, which increased bacterial counts of few strains by 0.18-0.89 log_{10} units after 8/24-h incubation at 37ºC (Table 4).

3.4. Analysis of stress/tolerance gene expression in oil-adapted L. pentosus strains

qRT-PCR was used to evaluate the differences betwen oil-adapted *L. pentosus* strains (which showed an increase bile tolerance and/or acid resistance) and wild-type strains (non adapted) in their expression of stress/tolerance genes: *gapd*, *tuf*, *fus*, *prep*, *groEL*, *enol*, *adhes*, *pgm* and *rpsL*. Firstly, screening of *L. pentosus* strains for all nine genes by conventional PCR was done, and the results (data not shown) showed only eight genes detected in all *L. pentosus* strains, while the *adhes* gene was only detected in two strains: *L. pentosus* MP-10 and *L. pentosus* CF2-10N.

Differential expression analyses (RT-qPCR) revealed that *fus*, *rpsL, groEL* and *pgm* became over-induced in oil-adapted *L. pentosus* strains (Fig. 3); however, the repertoire of genes induced in oil-adapted strains differed from each other. Genes *fus*, *rpsL* and *pgm* of *L. pentosus* CF1-6 were over-expressed in sunflower- and argan-adapted cells that became bile-tolerant (Fig. 3A). Furthermore, *gapd* and *fus* genes were over-expressed in sunflower- (bile tolerant) and olive-adapted (bile and acid tolerant) *L. pentosus* CF1-6, respectively (Fig. 3A). However, *L. pentosus* CF2-12 revealed that *prep*, *enol, groEL*, *gapd* and *rpsL* genes became over-expressed in linseed-adapted cells, and only *prep* gene was over-expressed in olive-adapted cells; all aforementioned oil adaptations created bile tolerance in this strain (Fig. 3B). Regarding *L. pentosus* AP2-15, *groEL*, *enol, pgm* and *rpsL* genes were over-expressed in oil-adapted cells exhibiting acid resistance (Fig. 3C). However, *L. pentosus* AP2-16 adapted with sunflower or argan oils showed an increase in the expression of *rpsL, pgm*, and *fus* genes, and exhibited acid resistance (Fig. 3D). On the other hand, *L. pentosus* MP-10 adapted with olive oil exhibited bile tolerance, and their over-expressed genes were *groEL*, *enol*, *fus*, *pgm* and *rpsL*; however, adaptations with other oils such as soy, almond or argan induced an over-expression of *groEL*, *pgm* and *enol*, respectively in acid-tolerant cells (Fig. 3E). Concerning *L. pentosus* CF2-10N, *pgm, fus* and *rpsL* genes were over-expressed in olive or linseed-adapted cells, which exhibited bile tolerance (Fig. 3F). Furthermore, over-expressed *prep* gene was also observed in linseed-adapted cells that were bile tolerant (Fig. 3F). *L. pentosus* CF1-39 adapted with linseed, soy, corn or argan oils exhibited an over-expression of *gapd*, *prep*, *tuf*, and *enol*; adapted cells were acid-resistant (Fig. 3G).

Quantification of gene expression revealed that some genes were over-expressed up to 37-303 times when compared with controls: e.g., 9-61 fold change in argan-adapted *L. pentosus* CF1-6, 2-60 fold change in linseed-adapted *L. pentosus* CF2-12, 2-37 fold change in argan-adapted *L. pentosus* AP2-15, 11-70 in sunflower-adapted *L. pentosus* AP2-16, 23-303 fold change in olive-adapted *L. pentosus* MP-10, 3-95 fold change in linseed-adapted *L. pentosus* CF2-10 and 2-4 fold change in argan-adapted *L. pentosus* CF1-39 (Fig. 3).

4. Discussion

Vegetable edible oils have been proposed for centuries as food-grade ingredients, condiments, cosmetics and also as therapeutic agents due to their antimicrobial and/or anti-inflammatory activities (Gurib-Fakim, 2006; Riechart, 2002). Furthermore, vegetable oils have been used as components for emulsions carrying microorganisms, genes, antigenic proteins and drugs (Nam, et al., 2009; Ying et al., 2010). However, as a dietary component, little information remains available about their effects on probiotics and other healthy bacteria in food products and the gut (Shahdadi et al., 2015). To ensure the functionality of probiotics, microorganisms must remain viable throughout the shelf-life of the products, in which they are incorporated, and within the gastrointestinal tract (Galdeano & Perdigón, 2004). As such, vegetable edible oils added to probiotic foods, or as part of diet, may affect their viability and functionality; they constitute a source of potent natural biologically active agents unable to discriminate between beneficial and pathogenic bacteria (Nychas, et al. 2003). On the other hand, essential oils have been reported to inhibit pathogens, and against some probiotic bacteria (Mahmoudi, et al. 2014; Nychas, 1995). Taking into consideration these reports, the current study had two main goals: firstly, *in vitro* evaluation of how dietary oils affect the growth of probiotic bacteria, and secondly, how pre-adaptation with vegetable edible oils increase probiotic bacteria robustness and improved probiotic features.

In this study, the effect of edible oils on the growth of probiotic *L. pentosus* strains isolated from Aloreña green table olives was examined *in vitro*, since there is great interest in developing probiotic foods containing oils. As such, the viability (i.e., survivability and optimal growth) of probiotic cells must be ensured during food processing and storage, as well as within the gastrointestinal tract where they promote health benefits (Ranadheera, et al. 2010). This study indicates that the tested vegetable oils (at 2% as an adequate concentration to test all oils) promoted varying levels of growth inhibition of probiotic *L. pentosus*, and each probiotic *L. pentosus* strain responded differently although the cell counts were often greater than the minimum 10^8 CFU/ml requirement for a product to be considered probiotic. Furthermore, mint essential oil showed the greatest inhibitory effect when compared with the other oils (i.e., sunflower, olive, linseed, soy, corn, almond and argan), decreasing bacterial viability up to 1.2 log_{10} units following a 24-h incubation, with cell counts in most cases remaining $>10^8$ CFU/ml. In a similar manner, Moritz, et al. (2012) reported that mint essential oil only caused sublethal stress to a probiotic *L. rhamnosus* in fermented milk during its shelf-life period; however, Shahdadi, et al. (2015) indicated that mint essential oil decreased the viability of probiotic *L. acidophilus* and inhibited pH reduction during the storage of drinking yoghurt. The fatty acids, present as triglycerides in these oils, and polyphenols directly inhibit the viability of probiotic bacteria depending on the type of oil and reactions by the exposed strain. Here, both the growth and capacity to acidify were relatively affected by the oils treatments, especially by mint essential oil, although they often did not decrease cell viabilities below the minimum count required to be considered a probiotic. In light of these findings, the ingestion of some oils may affect the viability of some beneficial bacteria, but could aid in the reduction of pathogens in both food products and the gut.

Considering that the viability of probiotic bacteria and their functionality depend on the strain and the oil used, second-generation probiotics were obtained by pre-adaptating probiotic *L. pentosus* strains with the different oils. The use of second-generation probiotics may have additional positive effects, inluding enhanced probiotic activities compared to the parental *L. pentosus* strains. The results showed improved growth rate of adapted bacteria (versus non-adapted bacteria) once exposed to oils, reaching similar or greater viable counts (up to 9 log_{10} units) than controls grown in the absence of oils. In this sense, probiotic bacteria respond to stress by producing specific substances, such as exopolysaccharides and proteins which may protect cells from further stressors (Nguyen, et al., 2016). Furthermore, this adaptation had a great impact on their probiotic features as detected *in vitro*, such as tolerance to low pH and bile salts. On the other hand, it has been widely reported that probiotic features are highly linked to strain and their produced substances; however, exposure conditions to the probiotic strain are crucial to determine their functionality such as responses to different environmental (including gastrointestinal) or technological stresses. Pre-exposing probiotic bacteria to stress can affect their robustness as reported previously by Casado Muñoz et al. (2016), which indicated that pre-exposure of probiotic *L. pentosus* to acids enhanced probiotic functions such as auto-aggregation via surface proteins. Other studies revealed that probiotics pre-adapted to multiple stress factors such as acids, bile or temperature are more robust under simulated gastrointestinal conditions than their parental counterparts, and exhibit enhanced antagonistic actions against pathogens (Mathipa & Thantsha, 2015). Following on these studies, the survivability of oil-adapted *L. pentosus* strains under low pH and high bile concentration was compared; the results demonstrated that pre-adaptation of probiotics with some oils improved their acid and bile tolerance. Acid tolerance of the non-adapted and the adapted *L. pentosus* strains was similar at pH 2.0, however evident differences were detected at pH 1.5, depending on the oil used and the strain tested. Overall, corn, argan, sunflower and soy most effectively induced acid tolerance in almost all *L. pentosus* strains, followed by olive, almond and lineseed oils. However, olive, linseed and argan oils increased bile tolerance in most *L. pentosus* strains. These results suggest that different mechanisms were used to withstand both stresses applied in this study.

To gain a greater insight into molecular mechanisms involved in acid/bile tolerance after oil adaptation, the expression of genes involved in stress/tolerance response was compared. Previous studies (e.g., Pérez Montoro et al., 2016), using comparative proteomic analysis, determined that the protein markers involved in acid resistance in *L. pentosus* were 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase 2 (PGAM-d) and elongation factor

G, which were both over-produced under standard and acidic conditions. As such, analyses of *pgm*, coding for phosphoglycerate mutase; *fus*, coding for elongation factor G; and other genes such as *gapd*, coding for glyceraldehyde-3-phosphate dehydrogenase; *tuf*, coding for elongation factor Tu; *prep*, coding for prepilin; *groEL*, coding for heat shock protein GroEL; *enol*, coding for enolase; *adhes*, coding for adhesin; and *rpsL*, coding for 30S ribosomal subunit protein S12 revealed that oil-adapted *L. pentosus* strains exhibited a different repertoire of gene over-expression, depending on the strain and the oil used for adaptation. Comparing with the parental strains, the adaptive responses of each *L. pentosus* strain was related with different sets of genes (i.e., *groEL*, *pgm, rpsL, fus*, *gapd*, *tuf*, *prep*, and *enol*) over-expressed to maintain intracellular pH homeostasis, energy production, protein and carbohydrate metabolism, and secretion. In each adapted *L. pentosus* strain, depending on the oil used, a balance of different responses was involved in tolerance/resistance which is a stable and irreversible trait. Regarding bile tolerance, different sets of genes (*fus*, *pgm, gapd*, *prep*, *groEL*, *enol* and *rpsL*) were over-expressed. Overall, independently of the strain and the oil treatment, *fus*, *rpsL, pgm, groEL, enol* and *prep* genes were over-induced in oil-adapted *L. pentosus* strains involved in acid/bile tolerance. The response to oils especially olive, argan, sunflower and linseed oils triggered the induction of genes involved in metabolism to ensure survival under oil stress, and consequently, they were also involved in acid and/or bile tolerance. Pérez-Montoro et al. (2016) reported that *L. pentosus* strains pre-exposed to acids displayed better probiotic function, including increased auto-aggregation ability, by means of moonlighting proteins such as elongation factor G (encoded by *fus* gene) and 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase 2 (encoded by *pgm* gene). As such, both genes coding for moonlighting proteins, which were involved in acid tolerance, were also induced by oils. Furthermore, Pérez Montoro et al. (2018) found that the genes coding for some of the biomarker proteins involved in mucin adhesion of *L. pentosus* were also induced by oils; thus, we can suggest that this pre-adaptation may be involved also in improving the adhesion ability of probiotic *L. pentosus* in the gut besides their acid tolerance.

5. Conclusions

This study's novelty lies in the fact that it investigated whether probiotic *L. pentosus* strains of vegetable origin could become affected by vegetable edible oils, and further how pre-exposure to such oils contribute to their robustness. Pre-adaptation of probiotic *L. pentosus* strains with oils constitute a possible new strategy to: 1) increase their viability and growth, 2) their capacity to withstand several stresses such as acids or bile in food products/gut, and also 3) to improve their functional properties as a probiotic. Pre-adaptation with olive, argan, sunflower and linseed oils induced the expression of genes (i.e., *fus*, *rpsL, pgm, groEL, enol* and *prep*) coding for moonlighting proteins that are involved in several stress responses and other functions. Furthermore, pre-adaptation with oils may represent a new approach for probiotic product manufacture, thus improving the stability of bacteria during industrial processing that often risk compromising the viability and functionality of the strains.

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Table 1. Primers and PCR conditions used in this study.

Gene	Primer	Sequence (5´-3´)	Annealing Temperature (ºC)	PCR product size (bp)	Reference
gapd	*gapd-F* *gapd-R*	TCAAGAAGCATACTGAAGG TATCGTACCAAGCAACAGTC	52	165	This study
tuf	*tuf-F* *tuf-R*	TCCACAATTCTACTTCCACAC TATGACCACCTTCACGAACC	58	176	This study
fus	*fus-F* *fus-R*	AGGTTTGAAGGAAGCTATGG TTCCATACCTTCGATGTTACC	58	274	This study
prep	*prep-F* *prep-R*	TACAATCTAGTC TAGTTGAAG AGCACTGCAGGTGTAATGA	55	172	This study
groEL	*groEL-F* *groEL-R*	TTACAAGAACGTTTAGCTA ATGCAGCAACGTCTTTGA	50	187	This study
enol	*enol-F* *enol-R*	AGTACCCAATCGTTTCCAT AAGGTAGTCCGTGTTCGTA	51	134	This study
adhes	*adhes-F* *adhes-R*	AATCACGATACGACCGCA ATTGACAACTGTTGCCCA	51	176	This study
pgm	*pgm-F* *pgm-R*	ATGGCGCAATTTTCAATTTACT AGCCGTAGAAGACTTCCCG	54	274	Pérez Montoro et al. (2018)
rpsL	*rpsMP-10-Fw* *rpsMP-10-Rv*	ATTAATTCGTAAAGGCCGT ACTTCCGTAAAGCCGAGTTA	55	176	Casado Muñoz et al. (2016)
pheS	*pheS-21F* *pheS-23R*	CAYCCNGCHCGYGAYATGC GGRTGRACCATVCCNGCHCC	60	411	Naser et al. (2005)

Table 2. Growth of oil-adapted *Lactobacillus pentosus* strains in the presence of different oils.

Oil-adapted strains[§]		0 h	Growth in presence of oils (24 h)*								
		Control	Control (no oil)	Sunflower (SF)	Olive (O)	Linseed (L)	Soya (SY)	Corn (C)	Almonds (AL)	Argan (AR)	Mint (M)
L. pentosus CF1-6	SF	7.6 ± 0.0	9.0 ± 0.02^{e}	8.7 ± 0.03^{bcde}	8.5 ± 0.06^{bc}	8.9 ± 0.07^{de}	8.3 ± 0.18^{b}	8.6 ± 0.18^{bcd}	8.7 ± 0.03^{cde}	8.9 ± 0.08^{de}	7.8 ± 0.10^{a}
	O	7.8 ± 0.16	9.3 ± 0.11^{d}	8.8 ± 0.05^{b}	9.0 ± 0.03^{bc}	9.0 ± 0.12^{bc}	8.9 ± 0.21^{bc}	9.1 ± 0.12^{c}	9.1 ± 0.03^{cd}	9.0 ± 0.07^{bc}	7.7 ± 0.08^{a}
	L	6.7 ± 0.12	9.5 ± 0.05^{f}	9.4 ± 0.02^{e}	9.1 ± 0.10^{cd}	9.1 ± 0.01^{c}	9.0 ± 0.15^{b}	9.2 ± 0.06^{d}	9.2 ± 0.03^{d}	9.2 ± 0.03^{cd}	8.0 ± 0.02^{a}
	SY	7.3 ± 0.04	9.1 ± 0.01^{bc}	9.0 ± 0.11^{b}	9.1 ± 0.03^{bc}	9.1 ± 0.03^{bc}	9.0 ± 0.17^{b}	9.2 ± 0.02^{c}	9.1 ± 0.11^{bc}	9.1 ± 0.05^{bc}	4.4 ± 0.03^{a}
	C	6.6 ± 0.16	9.3 ± 0.02^{c}	9.0 ± 0.04^{b}	9.1 ± 0.04^{b}	9.1 ± 0.03^{bc}	9.0 ± 0.04^{b}	9.0 ± 0.08^{b}	9.2 ± 0.06^{c}	9.1 ± 0.08^{bc}	7.6 ± 0.06^{a}
	AL	7.5 ± 0.21	9.2 ± 0.01^{bc}	9.1 ± 0.06^{bc}	9.1 ± 0.11^{bc}	9.0 ± 0.08^{b}	9.2 ± 0.01^{c}	9.1 ± 0.01^{bc}	9.2 ± 0.04^{bc}	9.2 ± 0.14^{bc}	8.3 ± 0.01^{a}
	AR	7.7 ± 0.06	9.0 ± 0.06^{bc}	8.9 ± 0.13^{b}	8.9 ± 0.09^{b}	9.0 ± 0.07^{bc}	9.0 ± 0.04^{bc}	9.1 ± 0.01^{cd}	9.2 ± 0.03^{de}	9.3 ± 0.02^{e}	8.3 ± 0.07^{a}
L. pentosus CF2-12	SF	6.4 ± 0.12	9.4 ± 0.02^{b}	9.9 ± 0.06^{d}	9.7 ± 0.16^{c}	9.7 ± 0.05^{c}	10.4 ± 0.03^{e}	9.4 ± 0.06^{b}	9.4 ± 0.06^{b}	9.6 ± 0.04^{c}	5.2 ± 0.07^{a}
	O	7.4 ± 0.05	9.3 ± 0.03^{c}	9.2 ± 0.03^{c}	9.2 ± 0.12^{bc}	9.0 ± 0.1^{b}	9.2 ± 0.04^{bc}	9.1 ± 0.05^{bc}	9.1 ± 0.10^{bc}	7.5 ± 0.15^{a}	7.6 ± 0.11^{a}
	L	7.4 ± 0.10	8.6 ± 0.09^{cd}	8.4 ± 0.05^{b}	8.8 ± 0.05^{de}	8.8 ± 0.08^{e}	8.7 ± 0.09^{cde}	8.6 ± 0.13^{c}	9.5 ± 0.08^{f}	8.7 ± 0.02^{cde}	7.2 ± 0.04^{a}
	SY	7.6 ± 0.04	10.8 ± 0.01^{c}	10.6 ± 0.02^{b}	11.0 ± 0.01^{d}	11.0 ± 0.01^{d}	11.2 ± 0.01^{e}	11.2 ± 0.01^{e}	11.3 ± 0.01^{e}	11.3 ± 0.01^{e}	6.4 ± 0.04^{a}
	C	7.4 ± 0.09	11.2 ± 0.01^{e}	11.1 ± 0.0^{de}	11.0 ± 0.03^{cd}	10.8 ± 0.07^{c}	11.6 ± 0.01^{f}	11.1 ± 0.0^{de}	9.1 ± 0.02^{b}	8.9 ± 0.14^{b}	6.6 ± 0.01^{a}
	AL	7.2 ± 0.09	9.1 ± 0.08^{e}	9.0 ± 0.07^{cde}	9.0 ± 0.15^{cde}	8.9 ± 0.09^{bc}	8.9 ± 0.06^{bc}	9.1 ± 0.08^{de}	8.7 ± 0.09^{b}	8.9 ± 0.05^{bcd}	7.9 ± 0.04^{a}
	AR	7.8 ± 0.18	9.3 ± 0.0^{ef}	9.0 ± 0.16^{cd}	8.8 ± 0.02^{b}	8.7 ± 0.15^{b}	9.1 ± 0.02^{de}	8.9 ± 0.10^{bc}	9.0 ± 0.14^{cd}	9.4 ± 0.08^{f}	7.2 ± 0.01^{a}
ntosus A	SF	7.3 ± 0.10	9.1 ± 0.08^{b}	9.0 ± 0.02^{ab}	9.1 ± 0.06^{ab}	9.1 ± 0.06^{ab}	9.0 ± 0.07^{ab}	9.1 ± 0.02^{ab}	9.0 ± 0.01^{a}	9.1 ± 0.10^{ab}	8.3 ± 0.06^{c}

	O	7.3 ± 0.05	8.7 ± 0.09^{b}	9.0 ± 0.01^{de}	8.9 ± 0.05^{cde}	8.8 ± 0.18^{bcd}	8.7 ± 0.12^{bc}	9.1 ± 0.10^{e}	8.9 ± 0.09^{bcde}	9.0 ± 0.14^{cde}	8.4 ± 0.10^{a}
	L	7.3 ± 0.07	8.8 ± 0.0^{abc}	8.9 ± 0.11^{bcd}	8.9 ± 0.01^{cd}	8.7 ± 0.05^{ab}	8.8 ± 0.07^{abc}	8.9 ± 0.13^{bcd}	9.0 ± 0.16^{d}	8.9 ± 0.09^{cd}	8.6 ± 0.19^{a}
	SY	7.3 ± 0.04	9.1 ± 0.05^{c}	9.2 ± 0.03^{c}	9.1 ± 0.11^{c}	8.8 ± 0.19^{b}	9.2 ± 0.06^{c}	9.2 ± 0.04^{c}	9.1 ± 0.06^{bc}	9.5 ± 0.12^{d}	6.1 ± 0.09^{a}
	C	7.1 ± 0.19	8.7 ± 0.14^{a}	9.6 ± 0.02^{d}	9.4 ± 0.11^{c}	8.6 ± 0.06^{a}	9.9 ± 0.02^{ef}	9.8 ± 0.03^{e}	9.0 ± 0.04^{b}	10 ± 0.07^{f}	9.3 ± 0.10^{c}
	AL	7.3 ± 0.14	9.3 ± 0.08^{d}	9.2 ± 0.09^{cd}	8.5 ± 0.15^{a}	9.2 ± 0.03^{cd}	9.0 ± 0.03^{bc}	8.9 ± 0.17^{b}	9.3 ± 0.09^{cd}	8.5 ± 0.09^{a}	8.7 ± 0.04^{ab}
	AR	7.5 ± 0.15	9.2 ± 0.01^{e}	9.7 ± 0.01^{g}	8.9 ± 0.07^{d}	8.6 ± 0.05^{c}	8.2 ± 0.08^{b}	8.2 ± 0.14^{b}	8.3 ± 0.03^{b}	9.5 ± 0.05^{f}	7.4 ± 0.07^{a}
***L. pentosus* AP2-16**	**SF**	7.3 ± 0.12	9.3 ± 0.01^{c}	9.1 ± 0.07^{b}	9.1 ± 0.05^{bc}	9.0 ± 0.09^{b}	9.2 ± 0.09^{bc}	9.1 ± 0.26^{b}	9.2 ± 0.09^{bc}	9.0 ± 0.04^{b}	6.9 ± 0.06^{a}
	O	7.3 ± 0.01	9.7 ± 0.02^{e}	8.8 ± 0.1^{d}	8.5 ± 0.03^{c}	8.8 ± 0.0^{d}	8.2 ± 0.03^{b}	8.2 ± 0.07^{b}	8.8 ± 0.12^{d}	8.7 ± 0.04^{cd}	7.5 ± 0.06^{a}
	L	7.3 ± 0.07	8.7 ± 0.02^{e}	8.6 ± 0.05^{de}	8.4 ± 0.15^{cd}	8.2 ± 0.12	8.7 ± 0.05	8.5 ± 0.03	8.4 ± 0.03	7.9 ± 0.03	6.0 ± 0.18
	SY	7.2 ± 0.02	9.2 ± 0.12^{d}	9.0 ± 0.15^{d}	8.7 ± 0.12^{c}	7.3 ± 0.07^{b}	7.2 ± 0.07^{b}	7.3 ± 0.06^{b}	7.3 ± 0.02^{b}	7.3 ± 0.01^{b}	6.9 ± 0.11^{a}
	C	7.6 ± 0.11	8.8 ± 0.13^{bc}	8.5 ± 0.08^{c}	8.7 ± 0.11^{bc}	8.9 ± 0.05^{c}	8.8 ± 0.04^{bc}	8.8 ± 0.06^{c}	8.8 ± 0.03^{c}	8.7 ± 0.12^{bc}	5.3 ± 0.01^{a}
	AL	7.4 ± 0.10	8.6 ± 0.17^{b}	8.9 ± 0.08^{cd}	8.7 ± 0.02^{bc}	9.1 ± 0.07^{d}	8.8 ± 0.05^{bcd}	8.6 ± 0.08^{b}	8.5 ± 0.20^{b}	8.9 ± 0.10^{cd}	5.9 ± 0.0^{a}
	AR	7.4 ± 0.06	8.6 ± 0.27^{bcd}	8.8 ± 0.04^{cd}	8.6 ± 0.20^{bcd}	8.4 ± 0.31^{b}	8.6 ± 0.10^{bcd}	8.8 ± 0.02^{bcd}	8.5 ± 0.01^{bc}	8.8 ± 0.06^{d}	5.7 ± 0.13^{a}
***L. pentosus* MP-10**	**SF**	7.7 ± 0.13	9.0 ± 0.03^{d}	8.9 ± 0.05^{cd}	8.8 ± 0.07^{bc}	8.6 ± 0.09^{b}	8.7 ± 0.05^{bc}	8.8 ± 0.04^{bc}	9.0 ± 0.14^{d}	8.9 ± 0.06^{cd}	7.3 ± 0.02^{a}
	O	8.0 ± 0.07	9.2 ± 0.05^{b}	9.4 ± 0.06^{c}	9.3 ± 0.12^{bc}	9.3 ± 0.04^{bc}	9.4 ± 0.03^{c}	9.4 ± 0.05^{bc}	9.3 ± 0.07^{bc}	9.4 ± 0.01^{c}	5.8 ± 0.07^{a}
	L	7.5 ± 0.20	8.9 ± 0.06^{bcd}	9.1 ± 0.07^{cd}	9.1 ± 0.07^{d}	8.9 ± 0.04^{bcd}	9.1 ± 0.06^{d}	8.7 ± 0.14^{b}	9.0 ± 0.02^{bcd}	8.8 ± 0.15^{bc}	6.6 ± 0.08^{a}
	SY	7.3 ± 0.16	8.9 ± 0.04^{bc}	9.1 ± 0.05^{cd}	9.1 ± 0.19^{bcd}	9.0 ± 0.05^{bcd}	9.1 ± 0.08^{bcd}	9.2 ± 0.09^{d}	8.8 ± 0.04^{b}	8.9 ± 0.09^{bcd}	6.3 ± 0.24^{a}
	C	7.4 ± 0.05	9.1 ± 0.04^{de}	9.1 ± 0.12^{e}	8.8 ± 0.28^{b}	8.8 ± 0.13^{bc}	9.1 ± 0.06^{de}	9.0 ± 0.05^{cde}	8.9 ± 0.00^{bcd}	9.1 ± 0.07^{de}	4.0 ± 0.05^{a}
	AL	7.5 ± 0.06	9.0 ± 0.15^{cd}	8.8 ± 0.07^{bc}	8.7 ± 0.14^{b}	8.8 ± 0.14^{bc}	9.0 ± 0.10^{cd}	9.1 ± 0.02^{d}	9.0 ± 0.06^{cd}	8.9 ± 0.05^{bcd}	7.5 ± 0.03^{a}

	AR	7.5 ± 0.03	9.2 ± 0.06^{d}	9.1 ± 0.12^{cd}	9.0 ± 0.03^{bcd}	9.0 ± 0.03^{bc}	9.1 ± 0.05^{bcd}	9.0 ± 0.10^{bc}	8.9 ± 0.08^{b}	9.1 ± 0.06^{cd}	7.0 ± 0.07^{a}
***L. pentosus* CF2-10**	**SF**	7.7 ± 0.00	9.1 ± 0.04^{b}	9.2 ± 0.04^{bcd}	9.2 ± 0.07^{bcd}	9.1 ± 0.04^{bc}	9.1 ± 0.14^{b}	9.3 ± 0.00^{d}	9.1 ± 0.03^{bc}	9.2 ± 0.04^{cd}	7.6 ± 0.05^{a}
	O	7.4 ± 0.17	8.8 ± 0.12^{b}	9.1 ± 0.09^{bcd}	8.8 ± 0.06^{b}	9.3 ± 0.08^{d}	9.0 ± 0.18^{bc}	9.2 ± 0.07^{d}	9.1 ± 0.14^{cd}	9.2 ± 0.02^{cd}	7.4 ± 0.12^{a}
	L	7.2 ± 0.21	9.2 ± 0.06^{c}	9.1 ± 0.04^{bc}	9.1 ± 0.12^{bc}	9.2 ± 0.07^{c}	9.1 ± 0.03^{bc}	9.3 ± 0.10^{c}	9.1 ± 0.15^{bc}	9.0 ± 0.03^{b}	7.3 ± 0.08^{a}
	SY	7.7 ± 0.17	9.2 ± 0.05^{b}	9.2 ± 0.04^{b}	9.2 ± 0.07^{bc}	9.2 ± 0.07^{bc}	9.4 ± 0.06^{c}	9.3 ± 0.07^{bc}	9.2 ± 0.01^{bc}	9.2 ± 0.06^{bc}	7.4 ± 0.05^{a}
	C	7.4 ± 0.04	9.2 ± 0.04^{b}	9.0 ± 0.17^{b}	9.1 ± 0.04^{b}	9.0 ± 0.03^{b}	9.1 ± 0.04^{b}	9.0 ± 0.06^{b}	9.0 ± 0.08^{b}	9.0 ± 0.03^{b}	8.7 ± 0.07^{a}
	AL	7.6 ± 0.07	9.3 ± 0.06^{c}	9.2 ± 0.18^{bc}	9.2 ± 0.06^{bc}	9.1 ± 0.02^{b}	9.2 ± 0.03^{bc}	9.3 ± 0.10^{c}	9.1 ± 0.03^{b}	9.3 ± 0.03^{c}	6.1 ± 0.07^{a}
	AR	7.5 ± 0.12	9.1 ± 0.04^{de}	9.0 ± 0.09^{cd}	9.0 ± 0.07^{cd}	9.2 ± 0.13^{ef}	9.1 ± 0.06^{de}	9.3 ± 0.08^{f}	8.2 ± 0.02^{b}	8.8 ± 0.06^{c}	6.2 ± 0.03^{a}
***L. pentosus* CF1-39**	**SF**	7.7 ± 0.17	9.1 ± 0.02^{bc}	9.1 ± 0.11^{bc}	9.2 ± 0.03^{c}	9.2 ± 0.02^{c}	9.1 ± 0.01^{bc}	9.1 ± 0.06^{c}	9.0 ± 0.10^{b}	9.2 ± 0.03^{c}	7.6 ± 0.04^{a}
	O	7.6 ± 0.13	9.0 ± 0.16^{bc}	9.1 ± 0.06^{bc}	9.1 ± 0.06^{bc}	9.3 ± 0.02^{c}	9.3 ± 0.01^{c}	9.2 ± 0.04^{bc}	9.2 ± 0.05^{bc}	9.0 ± 0.04^{b}	7.7 ± 0.22^{a}
	L	7.7 ± 0.06	9.0 ± 0.17^{bc}	9.0 ± 0.04^{c}	8.8 ± 0.04^{b}	9.1 ± 0.05^{c}	9.0 ± 0.02^{c}	9.0 ± 0.03^{c}	9.0 ± 0.11^{c}	9.0 ± 0.09^{c}	6.9 ± 0.06^{a}
	SY	7.6 ± 0.11	8.9 ± 0.13^{b}	8.9 ± 0.02^{b}	8.8 ± 0.16^{b}	8.7 ± 0.15^{b}	8.9 ± 0.02^{b}	8.8 ± 0.10^{b}	8.7 ± 0.22^{b}	8.8 ± 0.04^{b}	6.9 ± 0.09^{a}
	C	7.7 ± 0.09	8.3 ± 0.03^{b}	9.1 ± 0.07^{de}	9.0 ± 0.11^{cde}	8.9 ± 0.06^{cd}	9.2 ± 0.06^{e}	9.0 ± 0.08^{cde}	8.9 ± 0.05^{cd}	8.8 ± 0.06^{c}	3.0 ± 0.00^{a}
	AL	7.7 ± 0.05	9.2 ± 0.06^{b}	9.1 ± 0.04^{b}	9.2 ± 0.02^{b}	9.1 ± 0.08^{b}	9.1 ± 0.22^{b}	9.1 ± 0.07^{b}	9.1 ± 0.08^{b}	9.2 ± 0.08^{b}	5.9 ± 0.01^{a}
	AR	7.4 ± 0.10	9.1 ± 0.03^{cde}	9.0 ± 0.07^{cd}	9.0 ± 0.08^{c}	9.1 ± 0.08^{cde}	9.2 ± 0.07^{de}	9.0 ± 0.06^{cd}	8.2 ± 0.06^{b}	9.2 ± 0.09^{e}	5.7 ± 0.02^{a}

Numbers represent log_{10} values, their mean +/- standard deviations (±SD).

*: Different lowercase letters represent significant differences according to 2-sided Tukey's HSD between strains (p <0.05).

§: Oil-adapted *L. pentosus* strains with sunflower oil (SF), olive oil (O), linseed (L), soya (SY), corn (C), almonds (AL), argan (AR).

Table 3. Viable counts of oil-adapted *L. pentosus* strains after exposure to acidic and standard conditions.

Strains	**Viability of oil-adapted *L. pentosus* strains (Log_{10} CFU/ml)***			
	Oil-adapted strains§	**pH 1.5**	**pH 2**	**PBS**
***L. pentosus* CF1-6**	**Control**	0.00 ± 0.00^{a}	9.00 ± 0.00^{a}	9.16 ± 0.09^{c}
	Sunflower	0.00 ± 0.00^{a}	9.08 ± 0.00^{a}	8.60 ± 0.00^{a}
	Olive	3.71 ± 0.11^{cd}	9.06 ± 0.15^{a}	9.08 ± 0.09^{bc}
	Linseed	4.46 ± 0.06^{d}	8.92 ± 0.15^{a}	9.18 ± 0.09^{c}
	Soy	1.53 ± 1.33^{b}	9.02 ± 0.10^{a}	9.14 ± 0.10^{c}
	Corn	1.53 ± 1.33^{b}	9.08 ± 0.00^{a}	8.99 ± 0.05^{bc}
	Almonds	3.08 ± 0.07^{c}	8.99 ± 0.09^{a}	9.11 ± 0.11^{bc}
	Argan	0.00 ± 0.00^{a}	9.04 ± 0.06^{a}	8.85 ± 0.11^{b}
***L. pentosus* CF2-12**	**Control**	1.63 ± 1.42^{b}	8.83 ± 0.02^{d}	9.22 ± 0.03^{c}
	Sunflower	0.00 ± 0.00^{a}	8.73 ± 0.07^{cd}	9.06 ± 0.08^{abc}
	Olive	0.00 ± 0.00^{a}	8.39 ± 0.08^{a}	8.96 ± 0.09^{a}
	Linseed	0.00 ± 0.00^{a}	8.53 ± 0.05^{b}	8.96 ± 0.09^{a}
	Soy	0.00 ± 0.00^{a}	8.33 ± 0.06^{a}	8.99 ± 0.05^{ab}
	Corn	0.00 ± 0.00^{a}	8.65 ± 0.10^{bc}	8.98 ± 0.05^{a}
	Almonds	0.00 ± 0.00^{a}	8.73 ± 0.03^{cd}	9.18 ± 0.03^{bc}
	Argan	0.00 ± 0.00^{a}	8.60 ± 0.11^{bc}	9.14 ± 0.04^{abc}
***L. pentosus* AP2-15**	**Control**	2.69 ± 0.12^{b}	9.22 ± 0.08^{d}	9.13 ± 0.08^{d}
	Sunflower	4.02 ± 0.10^{d}	8.90 ± 0.03^{b}	9.05 ± 0.02^{cd}
	Olive	4.60 ± 0.00^{e}	8.93 ± 0.03^{bc}	8.90 ± 0.02^{ab}
	Linseed	0.00 ± 0.00^{a}	8.77 ± 0.07^{a}	8.85 ± 0.01^{a}
	Soy	4.48 ± 0.09^{e}	8.88 ± 0.09^{b}	9.00 ± 0.01^{bc}
	Corn	4.55 ± 0.03^{e}	9.02 ± 0.12^{bc}	8.82 ± 0.03^{a}
	Almonds	3.99 ± 0.09^{d}	9.07 ± 0.10^{c}	8.85 ± 0.03^{a}
	Argan	3.37 ± 0.14^{c}	8.95 ± 0.04^{bc}	8.86 ± 0.02^{a}
***L. pentosus* AP2-16**	**Control**	4.84 ± 0.09^{d}	9.03 ± 0.05^{bc}	9.14 ± 0.04^{b}
	Sunflower	5.10 ± 0.08^{e}	9.02 ± 0.12^{bc}	9.06 ± 0.15^{ab}
	Olive	4.77 ± 0.04^{d}	8.94 ± 0.06^{b}	9.06 ± 0.08^{ab}
	Linseed	4.18 ± 0.09^{b}	9.18 ± 0.04^{c}	9.10 ± 0.07^{ab}
	Soy	4.07 ± 0.10^{b}	9.05 ± 0.08^{bc}	9.04 ± 0.12^{ab}
	Corn	4.52 ± 0.04^{c}	9.05 ± 0.08^{bc}	8.87 ± 0.03^{a}
	Almonds	3.74 ± 0.05^{a}	9.07 ± 0.10^{bc}	9.09 ± 0.11^{ab}
	Argan	5.15 ± 0.13^{e}	8.69 ± 0.12^{a}	8.94 ± 0.04^{ab}

***L. pentosus* MP-10**	**Control**	0.00 ± 0.00^{a}	9.07 ± 0.10^{ab}	9.25 ± 0.03^{c}
	Sunflower	0.00 ± 0.00^{a}	8.99 ± 0.12^{a}	8.99 ± 0.06^{ab}
	Olive	0.00 ± 0.00^{a}	9.20 ± 0.08^{abc}	9.22 ± 0.05^{bc}
	Linseed	0.77 ± 1.33^{a}	9.10 ± 0.14^{abc}	9.20 ± 0.03^{bc}
	Soy	5.48 ± 0.00^{c}	9.25 ± 0.10^{abc}	9.19 ± 0.14^{bc}
	Corn	6.45 ± 0.03^{d}	9.12 ± 0.07^{abc}	9.23 ± 0.03^{bc}
	Almonds	5.17 ± 0.03^{c}	9.32 ± 0.09^{c}	8.84 ± 0.04^{a}
	Argan	3.07 ± 0.10^{b}	9.29 ± 0.13^{bc}	9.18 ± 0.09^{bc}
***L. pentosus* CF2-10**	**Control**	0.00 ± 0.00^{a}	9.37 ± 0.02^{c}	9.12 ± 0.03^{ab}
	Sunflower	0.00 ± 0.00^{a}	9.14 ± 0.09^{b}	9.04 ± 0.11^{b}
	Olive	0.00 ± 0.00^{a}	8.89 ± 0.16^{a}	9.18 ± 0.03^{b}
	Linseed	0.00 ± 0.00^{a}	9.24 ± 0.06^{bc}	9.05 ± 0.04^{ab}
	Soy	0.00 ± 0.00^{a}	9.19 ± 0.11^{bc}	8.76 ± 0.10^{a}
	Corn	3.71 ± 0.20^{c}	9.16 ± 0.07^{b}	9.22 ± 0.05^{b}
	Almonds	0.00 ± 0.00^{a}	9.15 ± 0.15^{b}	8.60 ± 0.00^{a}
	Argan	2.45 ± 0.21^{b}	8.84 ± 0.09^{a}	9.09 ± 0.05^{ab}
L. pentosus CF1-39	**Control**	0.00 ± 0.00^{a}	8.94 ± 0.06^{bc}	9.05 ± 0.04^{a}
	Sunflower	0.93 ± 1.60^{a}	8.50 ± 0.17^{a}	9.14 ± 0.04^{ab}
	Olive	3.14 ± 0.12^{bc}	9.00 ± 0.20^{bcd}	9.09 ± 0.05^{a}
	Linseed	3.94 ± 0.06^{c}	9.18 ± 0.06^{d}	9.12 ± 0.06^{ab}
	Soy	4.10 ± 0.08^{c}	8.82 ± 0.07^{b}	9.28 ± 0.04^{c}
	Corn	2.45 ± 0.21^{b}	9.09 ± 0.15^{cd}	9.29 ± 0.03^{c}
	Almonds	0.00 ± 0.00^{a}	9.02 ± 0.12^{bcd}	9.07 ± 0.10^{a}
	Argan	2.69 ± 0.12^{b}	9.07 ± 0.12^{cd}	9.23 ± 0.06^{bc}

Numbers represent *log_{10}* values, their mean +/- standard deviations (±SD).

*: Different lowercase letters represent significant differences according to 2-sided Tukey's HSD between strains (p <0.05).

§: Oil-adapted *L. pentosus* strains with sunflower, olive, linseed, soya, corn, almonds and argan oils.

Control, non-adapted strain

Table 4. Viable counts of oil-adapted *L. pentosus* strains after exposure to bile salts.

Oil-adapted strains[§]		Viability of oil-adapted *L. pentosus* strains (Log_{10} CFU/ml) in the presence of different bile concentration*					
		1.8%			**3.6%**		
		0 h	**8 h**	**24 h**	**0 h**	**8 h**	**24 h**
L. pentosus CF1-6	**Control**	5.39 ± 0.03^{a}	5.83 ± 0.18^{c}	7.56 ± 0.05^{cd}	5.06 ± 0.08^{a}	5.20 ± 0.03^{abc}	7.18 ± 0.10^{b}
	Sunflower	5.39 ± 0.03^{a}	6.72 ± 0.17^{e}	7.73 ± 0.04^{e}	5.06 ± 0.08^{a}	5.80 ± 0.17^{e}	7.18 ± 0.05^{b}
	Olive	5.39 ± 0.03^{a}	6.36 ± 0.10^{d}	7.50 ± 0.10^{bc}	5.06 ± 0.08^{a}	5.47 ± 0.05^{d}	6.91 ± 0.17^{a}
	Linseed	5.39 ± 0.03^{a}	5.50 ± 0.17^{ab}	7.69 ± 0.09^{de}	5.06 ± 0.08^{a}	5.01 ± 0.20^{a}	7.22 ± 0.08^{bc}
	Soy	5.39 ± 0.03^{a}	5.76 ± 0.14^{c}	7.35 ± 0.02^{ab}	5.06 ± 0.08^{a}	5.19 ± 0.11^{abc}	7.24 ± 0.02^{bc}
	Corn	5.39 ± 0.03^{a}	5.39 ± 0.10^{a}	7.29 ± 0.15^{a}	5.06 ± 0.08^{a}	5.27 ± 0.09^{bcd}	7.19 ± 0.05^{bc}
	Almonds	5.39 ± 0.03^{a}	5.69 ± 0.09^{bc}	7.42 ± 0.06^{abc}	5.06 ± 0.08^{a}	5.09 ± 0.09^{ab}	7.15 ± 0.05^{b}
	Argan	5.39 ± 0.03^{a}	6.59 ± 0.11^{e}	7.60 ± 0.02^{cd}	5.06 ± 0.08^{a}	5.40 ± 0.17^{cd}	7.36 ± 0.06^{c}
L. pentosus CF2-12	**Control**	7.17 ± 0.15^{a}	7.16 ± 0.05^{abc}	7.62 ± 0.27^{bc}	7.14 ± 0.08^{a}	7.05 ± 0.10^{ab}	7.85 ± 0.05^{cd}
	Sunflower	7.17 ± 0.15^{a}	7.07 ± 0.10^{a}	7.46 ± 0.04^{ab}	7.14 ± 0.08^{a}	6.97 ± 0.12^{a}	7.79 ± 0.10^{c}
	Olive	7.17 ± 0.15^{a}	7.56 ± 0.07^{e}	7.94 ± 0.03^{e}	7.14 ± 0.08^{a}	7.63 ± 0.13^{d}	8.25 ± 0.13^{e}
	Linseed	7.17 ± 0.15^{a}	7.40 ± 0.03^{d}	7.80 ± 0.03^{cde}	7.14 ± 0.08^{a}	7.17 ± 0.02^{bc}	8.00 ± 0.06^{de}
	Soy	7.17 ± 0.15^{a}	7.14 ± 0.09^{abc}	7.44 ± 0.05^{ab}	7.14 ± 0.08^{a}	6.96 ± 0.16^{a}	7.67 ± 0.09^{bc}
	Corn	7.17 ± 0.15^{a}	7.19 ± 0.11^{bc}	7.32 ± 0.01^{a}	7.14 ± 0.08^{a}	7.06 ± 0.09^{abc}	7.02 ± 0.05^{a}
	Almonds	7.17 ± 0.15^{a}	7.10 ± 0.09^{ab}	7.83 ± 0.15^{de}	7.14 ± 0.08^{a}	6.96 ± 0.16^{a}	7.77 ± 0.10^{c}
	Argan	7.17 ± 0.15^{a}	7.24 ± 0.09^{c}	7.65 ± 0.04^{cd}	7.14 ± 0.08^{a}	7.24 ± 0.02^{c}	7.50 ± 0.15^{b}
L. pentosus AP2-15	**Control**	7.14 ± 0.02^{a}	7.39 ± 0.04^{bc}	7.78 ± 0.00^{bc}	7.08 ± 0.15^{a}	7.09 ± 0.09^{b}	7.44 ± 0.04^{ab}
	Sunflower	7.14 ± 0.02^{a}	7.41 ± 0.02^{bc}	7.84 ± 0.03^{bc}	7.08 ± 0.15^{a}	6.99 ± 0.12^{ab}	7.55 ± 0.02^{b}
	Olive	7.14 ± 0.02^{a}	7.31 ± 0.16^{ab}	7.99 ± 0.06^{c}	7.08 ± 0.15^{a}	7.07 ± 0.15^{ab}	7.51 ± 0.02^{ab}
	Linseed	7.14 ± 0.02^{a}	7.38 ± 0.11^{bc}	7.54 ± 0.04^{a}	7.08 ± 0.15^{a}	7.01 ± 0.15^{ab}	7.49 ± 0.06^{ab}
	Soy	7.14 ± 0.02^{a}	7.46 ± 0.04^{c}	7.92 ± 0.01^{c}	7.08 ± 0.15^{a}	7.08 ± 0.15^{b}	7.36 ± 0.03^{a}
	Corn	7.14 ± 0.02^{a}	7.34 ± 0.08^{abc}	7.52 ± 0.06^{a}	7.08 ± 0.15^{a}	7.23 ± 0.00^{b}	7.52 ± 0.07^{ab}
	Almonds	7.14 ± 0.02^{a}	7.22 ± 0.10^{a}	7.57 ± 0.08^{a}	7.08 ± 0.15^{a}	6.90 ± 0.09^{a}	7.39 ± 0.02^{ab}
	Argan	7.14 ± 0.02^{a}	7.27 ± 0.01^{ab}	7.62 ± 0.08^{ab}	7.08 ± 0.15^{a}	7.01 ± 0.09^{ab}	7.38 ± 0.05^{ab}
L. pentosus AP2-16	**Control**	7.11 ± 0.10^{a}	7.56 ± 0.07^{ab}	7.24 ± 0.09^{a}	7.16 ± 0.11^{a}	7.51 ± 0.15^{b}	7.08 ± 0.07^{a}
	Sunflower	7.11 ± 0.10^{a}	7.77 ± 0.10^{b}	7.26 ± 0.08^{a}	7.16 ± 0.11^{a}	7.36 ± 0.02^{a}	7.15 ± 0.02^{a}
	Olive	7.11 ± 0.10^{a}	7.64 ± 0.19^{b}	7.31 ± 0.04^{a}	7.16 ± 0.11^{a}	7.50 ± 0.05^{b}	7.16 ± 0.07^{a}
	Linseed	7.11 ± 0.10^{a}	7.39 ± 0.12^{a}	7.25 ± 0.00^{a}	7.16 ± 0.11^{a}	7.51 ± 0.03^{b}	7.32 ± 0.10^{b}
	Soy	7.11 ± 0.10^{a}	7.74 ± 0.13^{b}	7.32 ± 0.00^{a}	7.16 ± 0.11^{a}	7.49 ± 0.04^{b}	7.11 ± 0.02^{a}
	Corn	7.11 ± 0.10^{a}	7.73 ± 0.05^{b}	7.16 ± 0.09^{a}	7.16 ± 0.11^{a}	7.49 ± 0.04^{b}	7.34 ± 0.02^{b}
	Almonds	7.11 ± 0.10^{a}	7.65 ± 0.07^{b}	7.19 ± 0.01^{a}	7.16 ± 0.11^{a}	7.52 ± 0.02^{b}	7.13 ± 0.03^{a}
	Argan	7.11 ± 0.10^{a}	7.68 ± 0.14^{b}	7.19 ± 0.13^{a}	7.16 ± 0.11^{a}	7.52 ± 0.02^{b}	7.11 ± 0.05^{a}

***L. pentosus* MP-10**	**Control**	7.22 ± 0.06^{a}	7.65 ± 0.16^{b}	7.89 ± 0.06^{ab}	7.18 ± 0.09^{a}	7.44 ± 0.04^{a}	8.04 ± 0.04^{abc}
	Sunflower	7.22 ± 0.06^{a}	7.36 ± 0.10^{a}	7.90 ± 0.07^{ab}	7.18 ± 0.09^{a}	7.44 ± 0.10^{a}	7.93 ± 0.10^{a}
	Olive	7.22 ± 0.06^{a}	7.52 ± 0.07^{ab}	8.05 ± 0.04^{c}	7.18 ± 0.09^{a}	7.43 ± 0.03^{a}	8.25 ± 0.03^{d}
	Linseed	7.22 ± 0.06^{a}	7.49 ± 0.09^{ab}	7.98 ± 0.15^{ab}	7.18 ± 0.09^{a}	7.41 ± 0.05^{a}	7.94 ± 0.13^{ab}
	Soy	7.22 ± 0.06^{a}	7.53 ± 0.11^{ab}	8.03 ± 0.02^{ab}	7.18 ± 0.09^{a}	7.42 ± 0.05^{a}	7.90 ± 0.05^{a}
	Corn	7.22 ± 0.06^{a}	7.52 ± 0.03^{ab}	7.81 ± 0.11^{a}	7.18 ± 0.09^{a}	7.43 ± 0.03^{a}	8.18 ± 0.04^{bc}
	Almonds	7.22 ± 0.06^{a}	7.48 ± 0.07^{ab}	7.88 ± 0.03^{ab}	7.18 ± 0.09^{a}	7.46 ± 0.08^{a}	7.94 ± 0.11^{ab}
	Argan	7.22 ± 0.06^{a}	7.38 ± 0.06^{a}	7.87 ± 0.19^{ab}	7.18 ± 0.09^{a}	7.38 ± 0.04^{a}	8.14 ± 0.09^{abc}
***L. pentosus* CF2-10**	**Control**	7.17 ± 0.10^{a}	7.80 ± 0.08^{bcd}	7.93 ± 0.10^{b}	7.36 ± 0.08^{a}	7.59 ± 0.16^{a}	7.89 ± 0.11^{a}
	Sunflower	7.17 ± 0.10^{a}	7.69 ± 0.12^{bc}	8.11 ± 0.02^{cd}	7.36 ± 0.08^{a}	7.95 ± 0.05^{cd}	8.36 ± 0.04^{cd}
	Olive	7.17 ± 0.10^{a}	7.83 ± 0.13^{cde}	8.26 ± 0.04^{e}	7.36 ± 0.08^{a}	7.90 ± 0.09^{bcd}	8.46 ± 0.08^{cd}
	Linseed	7.17 ± 0.10^{a}	7.94 ± 0.12^{de}	8.31 ± 0.11^{e}	7.36 ± 0.08^{a}	7.80 ± 0.04^{abc}	8.48 ± 0.01^{d}
	Soy	7.17 ± 0.10^{a}	7.65 ± 0.16^{bc}	7.69 ± 0.09^{a}	7.36 ± 0.08^{a}	7.65 ± 0.07^{ab}	7.98 ± 0.15^{ab}
	Corn	7.17 ± 0.10^{a}	7.98 ± 0.03^{e}	7.98 ± 0.02^{bc}	7.36 ± 0.08^{a}	8.03 ± 0.14^{d}	8.11 ± 0.07^{b}
	Almonds	7.17 ± 0.10^{a}	7.64 ± 0.06^{ab}	8.01 ± 0.06^{bc}	7.36 ± 0.08^{a}	7.64 ± 0.19^{a}	8.32 ± 0.05^{c}
	Argan	7.17 ± 0.10^{a}	7.47 ± 0.06^{a}	8.18 ± 0.10^{de}	7.36 ± 0.08^{a}	7.62 ± 0.15^{a}	8.07 ± 0.07^{b}
***L. pentosus* CF1-39**	**Control**	7.25 ± 0.06^{a}	7.48 ± 0.06^{b}	7.36 ± 0.02^{c}	7.18 ± 0.12^{a}	7.43 ± 0.02^{ab}	7.15 ± 0.06^{a}
	Sunflower	7.25 ± 0.06^{a}	7.37 ± 0.13^{ab}	7.22 ± 0.05^{bc}	7.18 ± 0.12^{a}	7.40 ± 0.12^{ab}	7.05 ± 0.13^{a}
	Olive	7.25 ± 0.06^{a}	7.49 ± 0.08^{b}	7.12 ± 0.13^{ab}	7.18 ± 0.12^{a}	7.49 ± 0.04^{b}	7.19 ± 0.09^{a}
	Linseed	7.25 ± 0.06^{a}	7.43 ± 0.02^{ab}	7.36 ± 0.01^{c}	7.18 ± 0.12^{a}	7.33 ± 0.12^{a}	7.09 ± 0.02^{a}
	Soy	7.25 ± 0.06^{a}	7.46 ± 0.14^{b}	7.23 ± 0.05^{bc}	7.18 ± 0.12^{a}	7.31 ± 0.04^{a}	7.13 ± 0.16^{a}
	Corn	7.25 ± 0.06^{a}	7.31 ± 0.08^{a}	7.23 ± 0.03^{bc}	7.18 ± 0.12^{a}	7.36 ± 0.09^{ab}	7.06 ± 0.03^{a}
	Almonds	7.25 ± 0.06^{a}	7.50 ± 0.03^{b}	7.19 ± 0.08^{abc}	7.18 ± 0.12^{a}	7.31 ± 0.10^{a}	7.02 ± 0.05^{a}
	Argan	7.25 ± 0.06^{a}	7.46 ± 0.05^{b}	7.04 ± 0.12^{a}	7.18 ± 0.12^{a}	7.33 ± 0.01^{a}	7.07 ± 0.06^{a}

Numbers represent log_{10} values, their mean +/- standard deviations (±SD).

*: Different lowercase letters represent significant differences according to 2-sided Tukey's HSD between strains (p <0.05).

§: Oil-adapted *L. pentosus* strains with sunflower, olive, linseed, soya, corn, almonds and argan oils.

Figure legends

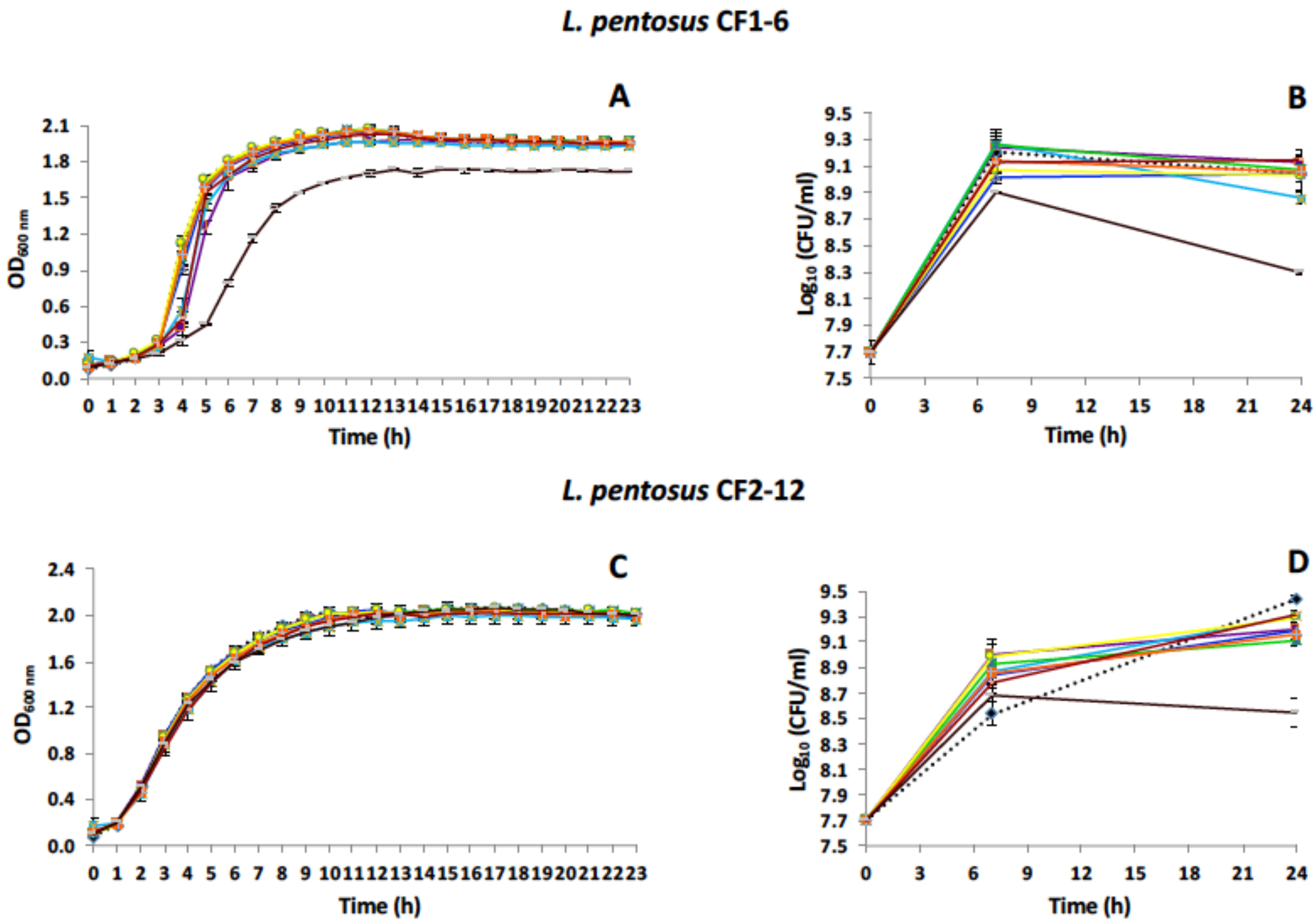
Figure 1. Viability of *L. pentosus* strains in the presence of edible oils and mint essential oil during incubation at 37ºC in MRS broth for 24 hours. Optical density at 600 nm was monitored (A, C, E, G, I, K and M) each hour, and the count of viable cells (CFU/ml) was determined (B, D, F, H, J, L and N) after 7 and 24 h for each strain. Values are expressed as the mean of the log_{10} (CFU/ml) of three independent experiments; error bars represent standard deviations.

Figure 2. Acidification capacity of *L. pentosus* strains grown in the presence of vegetable edible oils and mint essential oil in MRS broth at 37ºC for 24 hours. Significant differences ($p < 0.05$) in acidification capacity revealed by two-way ANOVA were dependent on the variable oil (A) and *L. pentosus* strain (B).

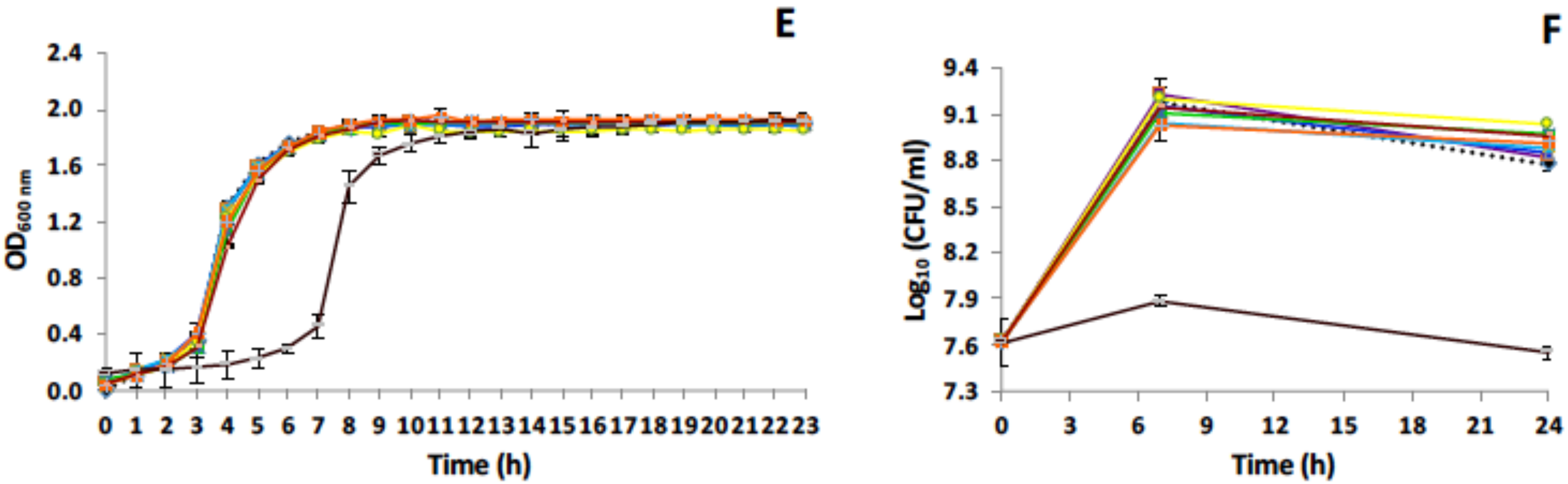
Figure 3. Analysis of the expression of *gapd*, *tuf*, *fus*, *prep*, *groEL*, *enol*, *adhes*, *pgm* and *rpsL* genes in oil-adapted *L. pentosus* strains. The relative expression level in control (non-adapted *L. pentosus* strains) was set to one for fold expression analysis in other experimental groups. Each bar represents mean value and standard deviation as error bar of three independent experiments. * denotes significant differences in gene expression between controls and oil-adapted *L. pentosus* strain ($P < 0.05$).

Supplementary Material

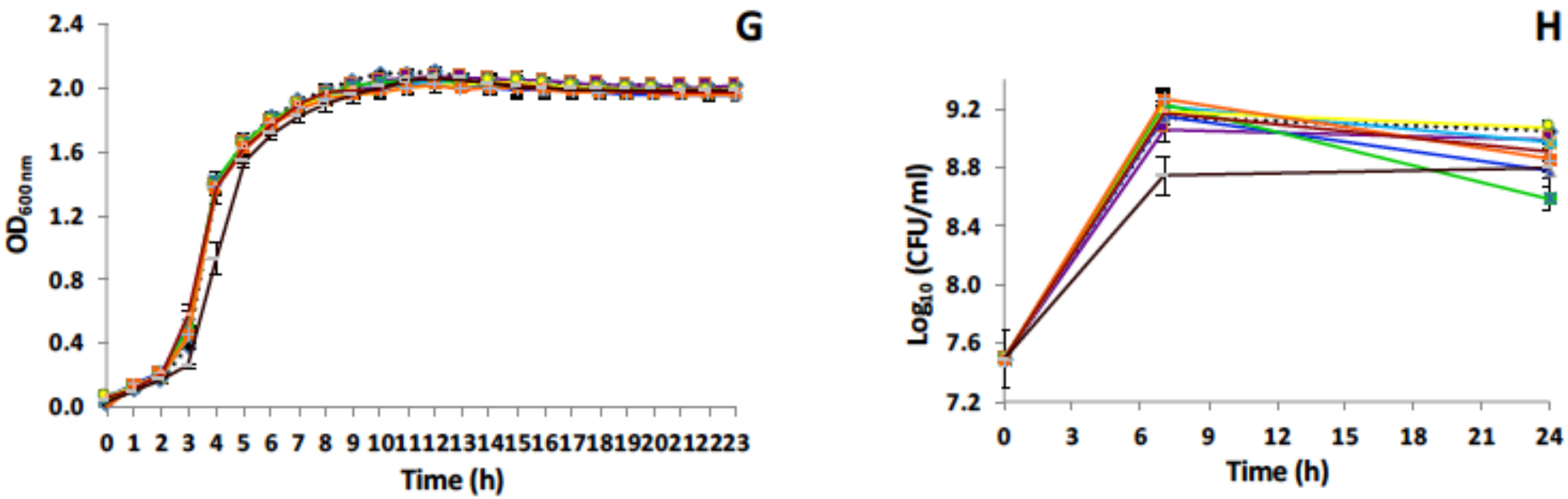
Figure S1. Viability of oil-adapted *L. pentosus* strains in MRS broth without oils during incubation at 37ºC for 24 hours. Optical density at 600 nm was monitored (A, C, E, G, I, K and M) each hour, and the count of viable cells (CFU/ml) was determined (B, D, F, H, J, L and N) after 7 and 24 h for each strain. Values are expressed as the mean of the log_{10} (CFU/ml) of three independent experiments; error bars represent standard deviations.


L. pentosus CF1-6
A
OD600 nm
Time (h)
B
Log10 (CFU/ml)
Time (h)
L. pentosus CF2-12
C
OD600 nm
Time (h)
D
Log10 (CFU/ml)
Time (h)

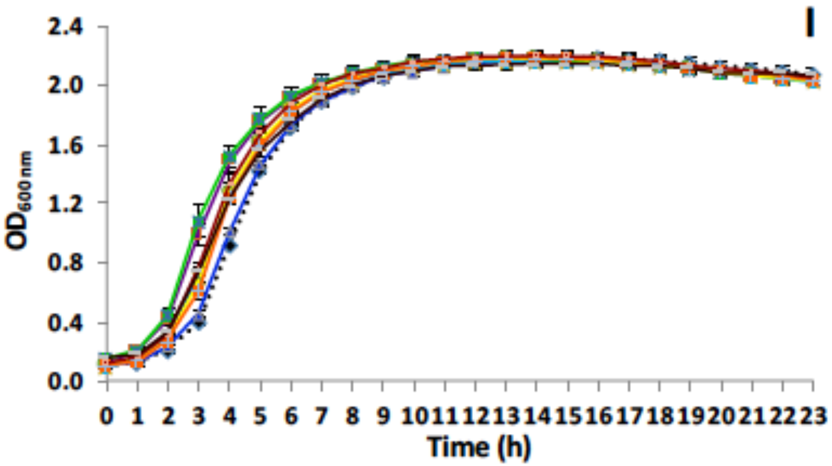
L. pentosus AP2-15

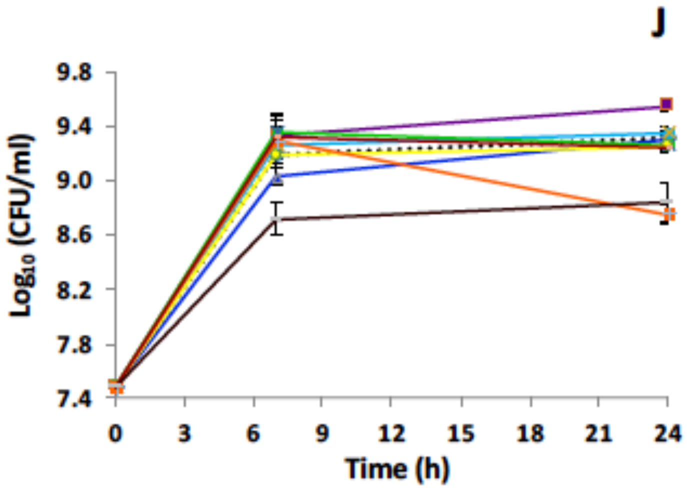


L. pentosus AP2-16

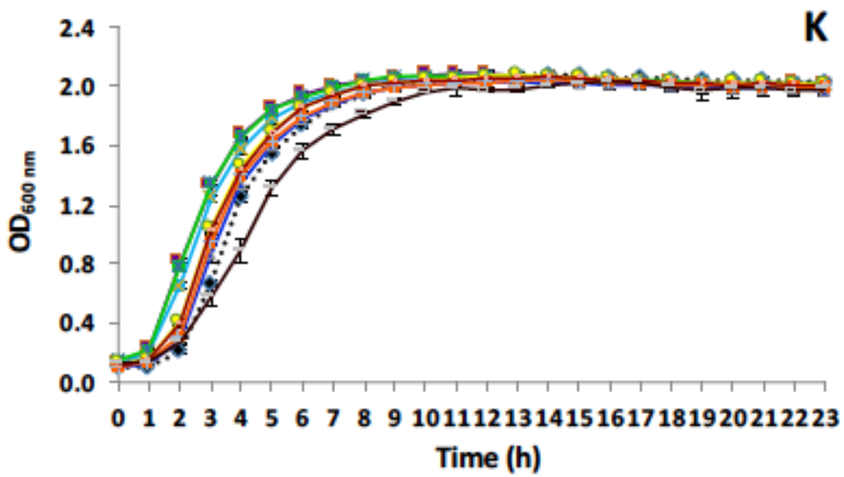


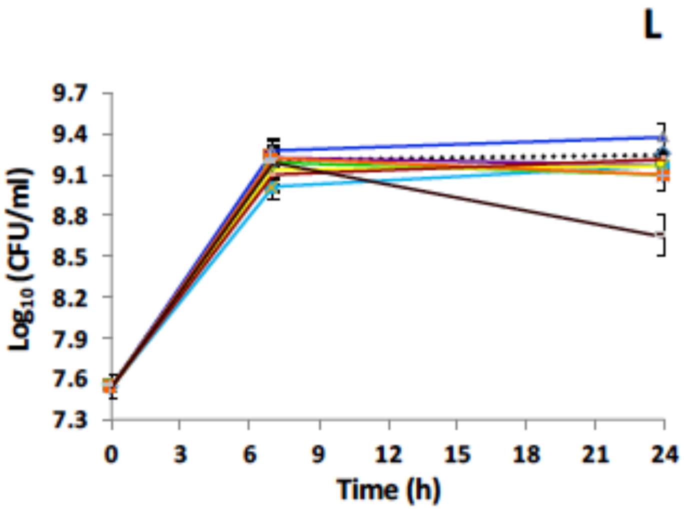
L. pentosus MP-10





L. pentosus CF2-10





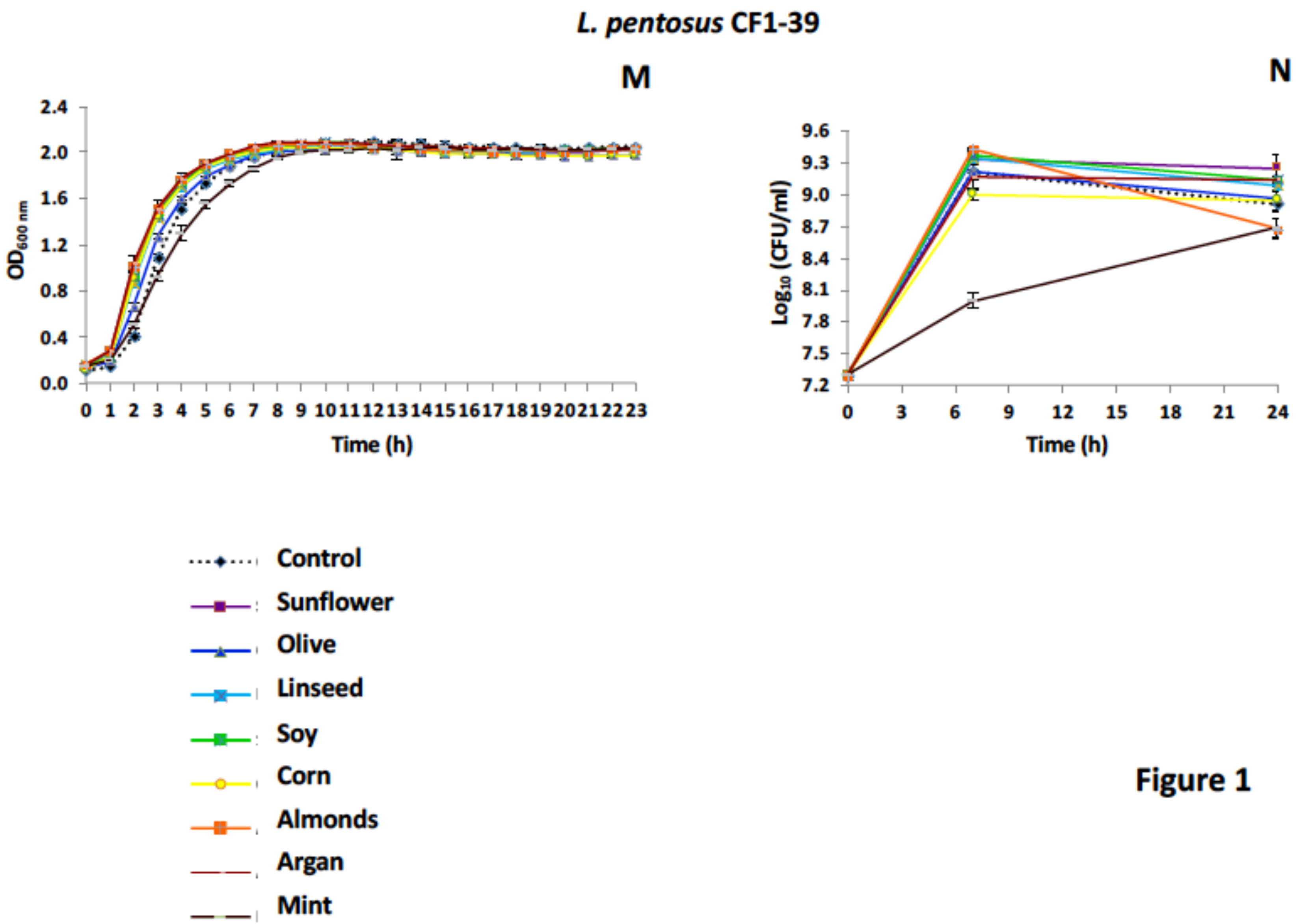
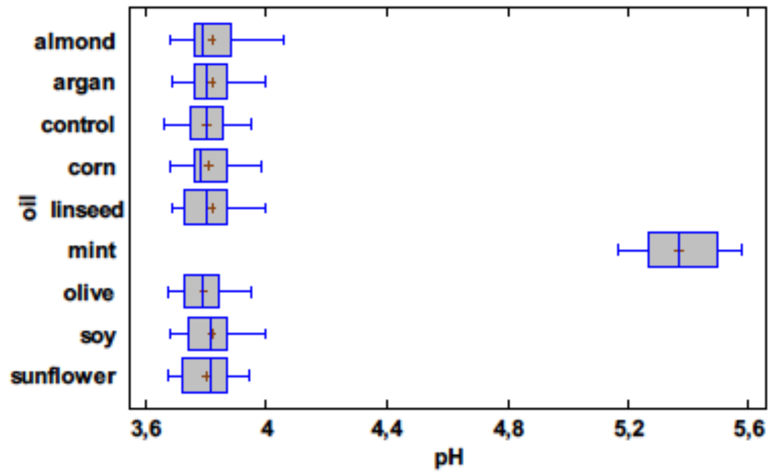

L. pentosus CF1-39
M
N
OD600 nm
Time (h)
Log10 (CFU/ml)
Time (h)
Control
Sunflower
Olive
Linseed
Soy
Corn
Almonds
Argan
Mint

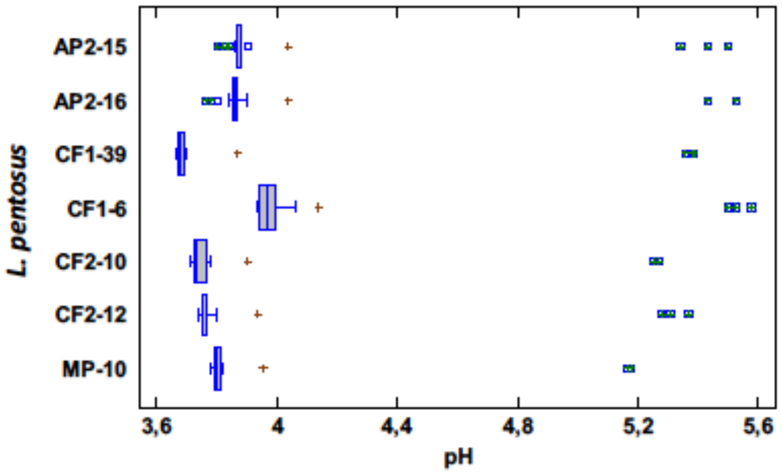
Figure 1

A



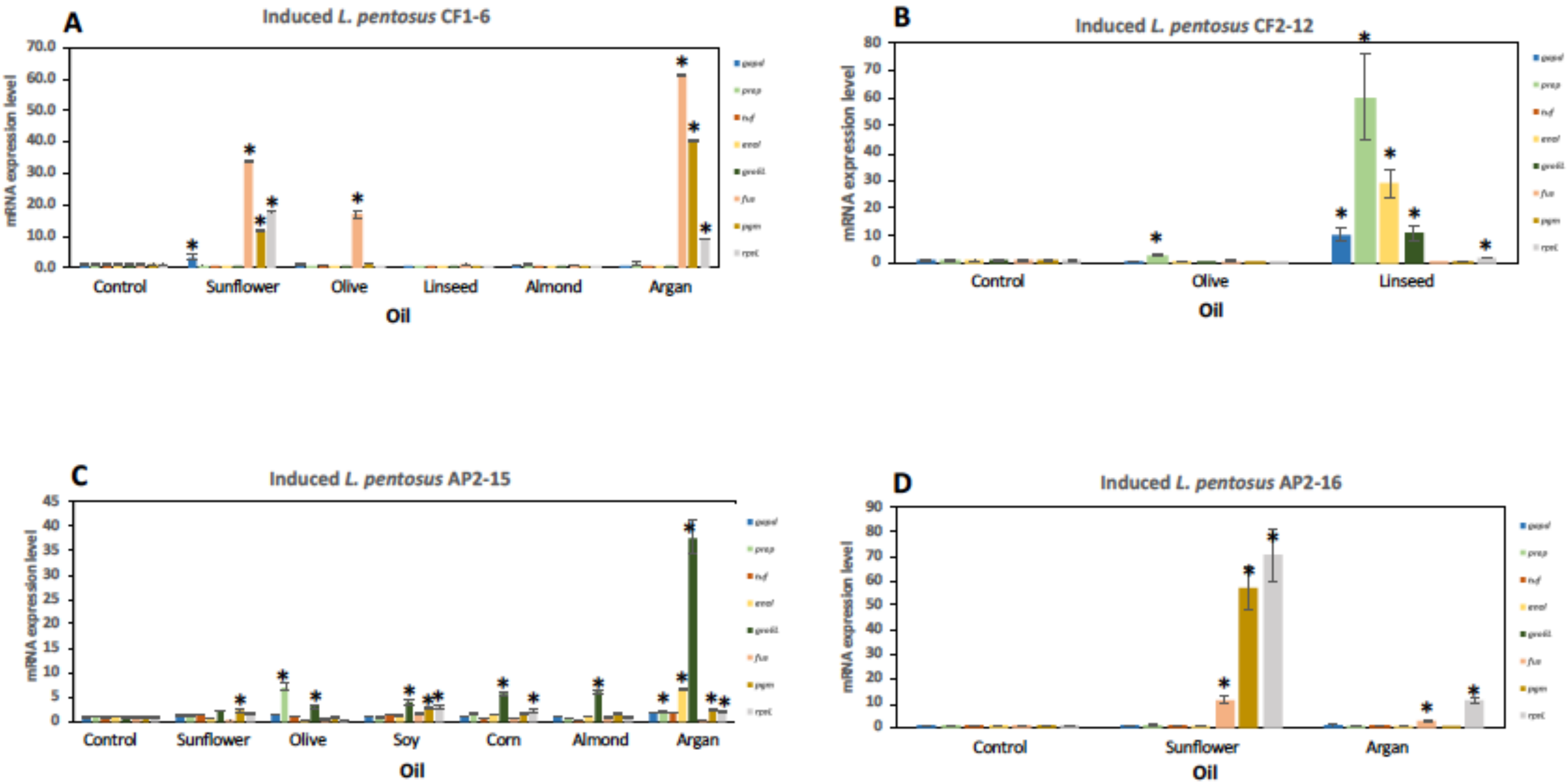
almond
argan
control
corn
linseed
mint
olive
soy
sunflower
oil
3,6
4
4,4
4,8
5,2
5,6
pH

B



AP2-15
AP2-16
CF1-39
CF1-6
CF2-10
CF2-12
MP-10
L. pentosus
3,6
4
4,4
4,8
5,2
5,6
pH

Figure 2


A
Induced L. pentosus CF1-6
mRNA expression level
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Control
Sunflower
Olive
Linseed
Almond
Argan
Oil
B
Induced L. pentosus CF2-12
mRNA expression level
80
70
60
50
40
30
20
10
0
Control
Olive
Linseed
Oil
C
Induced L. pentosus AP2-15
mRNA expression level
45
40
35
30
25
20
15
10
5
0
Control
Sunflower
Olive
Soy
Corn
Almond
Argan
Oil
D
Induced L. pentosus AP2-16
mRNA expression level
90
80
70
60
50
40
30
20
10
0
Control
Sunflower
Argan
Oil

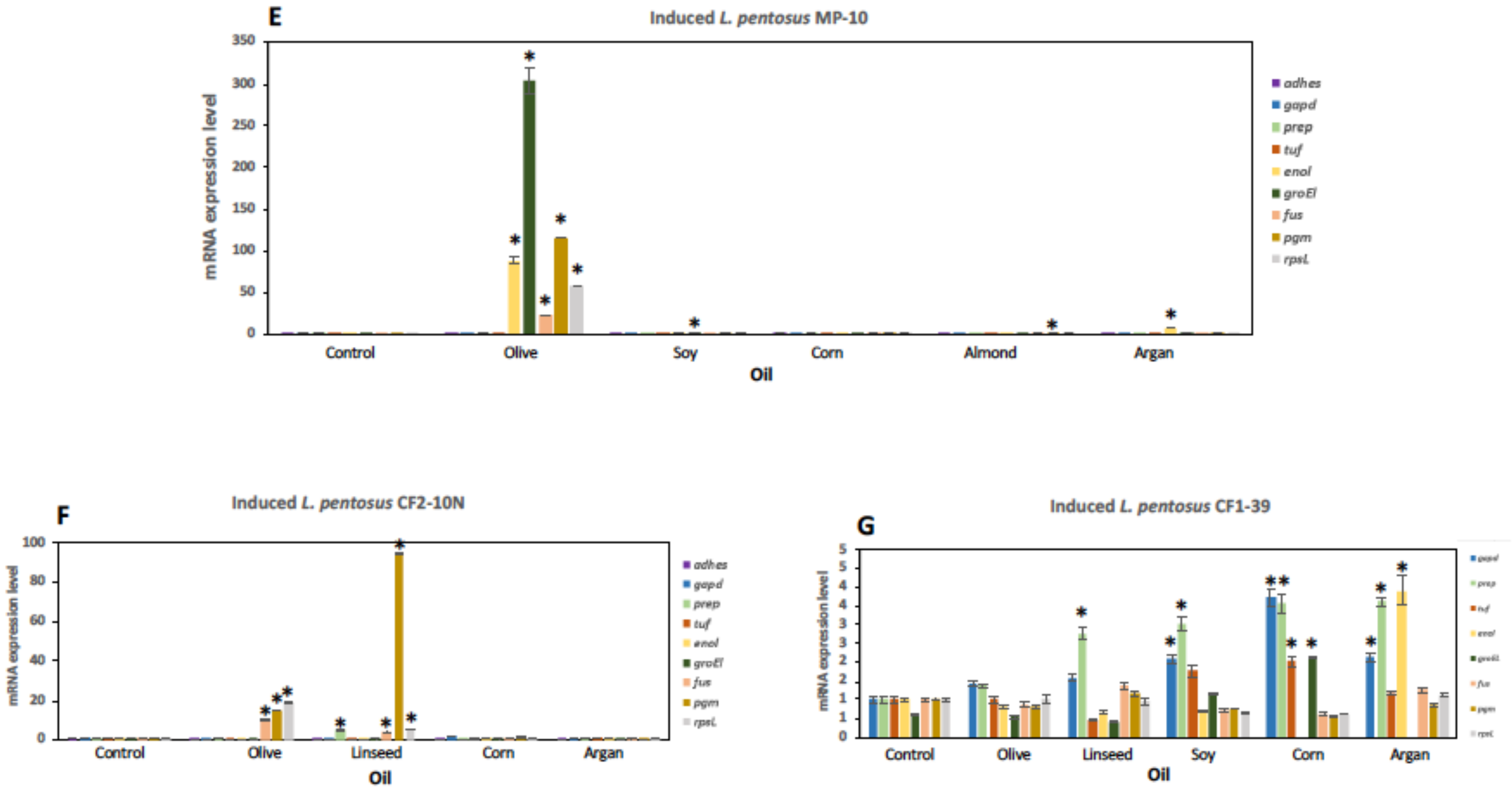
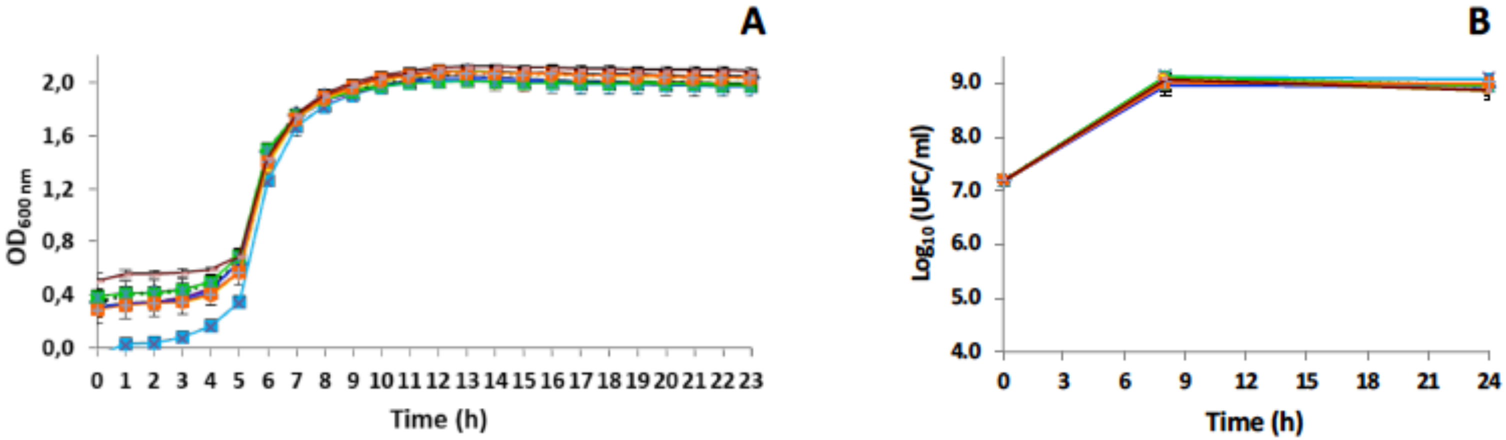
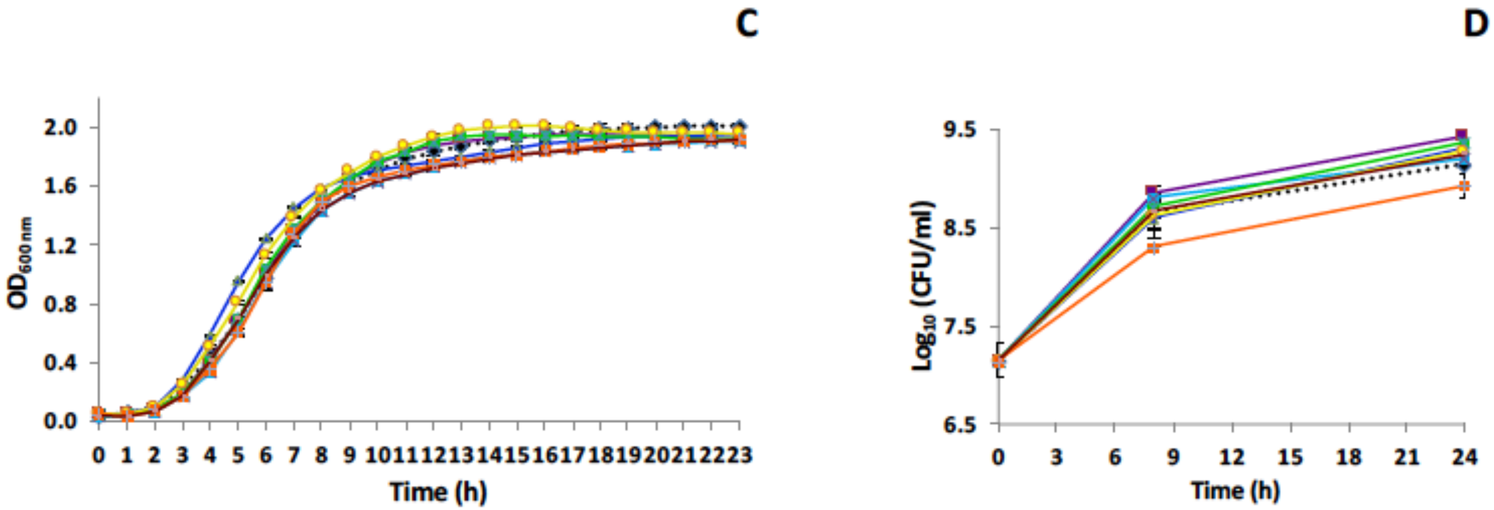

E
Induced L. pentosus MP-10
mRNA expression level
350
300
250
200
150
100
50
0
Control
Olive
Soy
Corn
Almond
Argan
Oil
adhes
gapd
prep
tuf
enol
groEl
fus
pgm
rpsL
F
Induced L. pentosus CF2-10N
mRNA expression level
100
80
60
40
20
0
Control
Olive
Linseed
Corn
Argan
Oil
adhes
gapd
prep
tuf
enol
groEl
fus
pgm
rpsL
G
Induced L. pentosus CF1-39
mRNA expression level
Control
Olive
Linseed
Soy
Corn
Argan
Oil

Figure 3

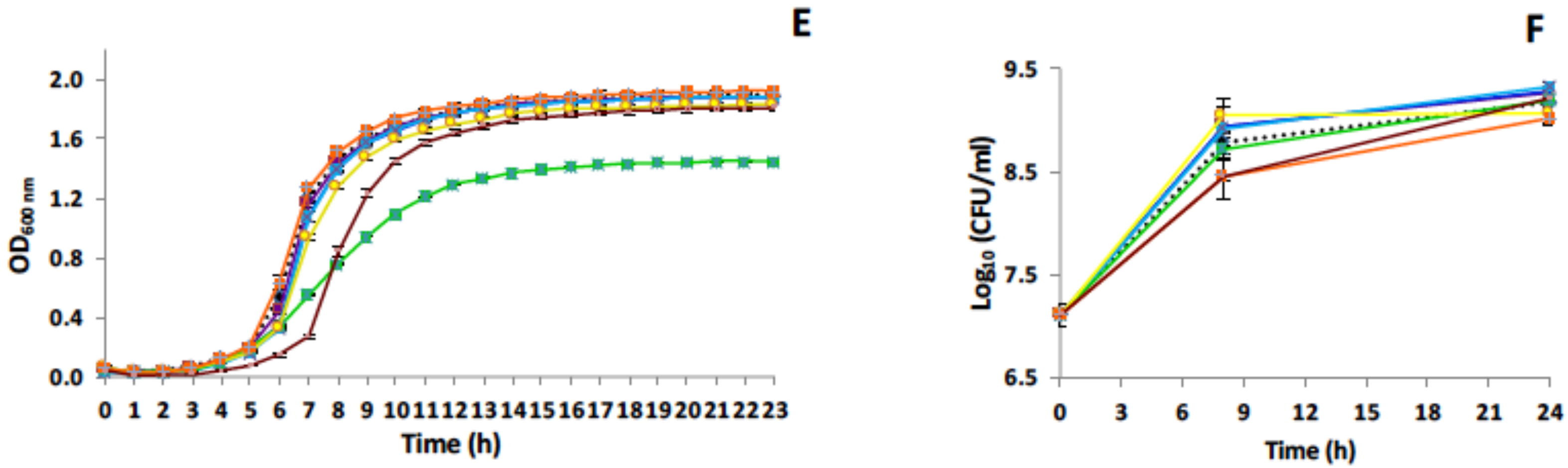
L. pentosus CF1-6



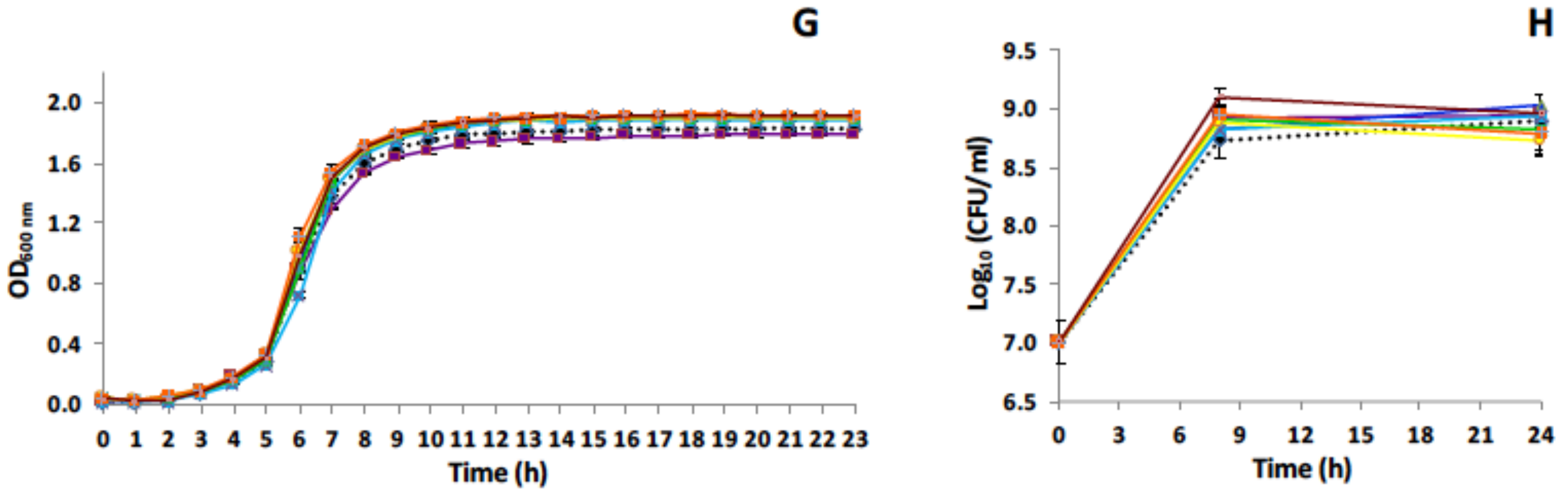
L. pentosus CF2-12



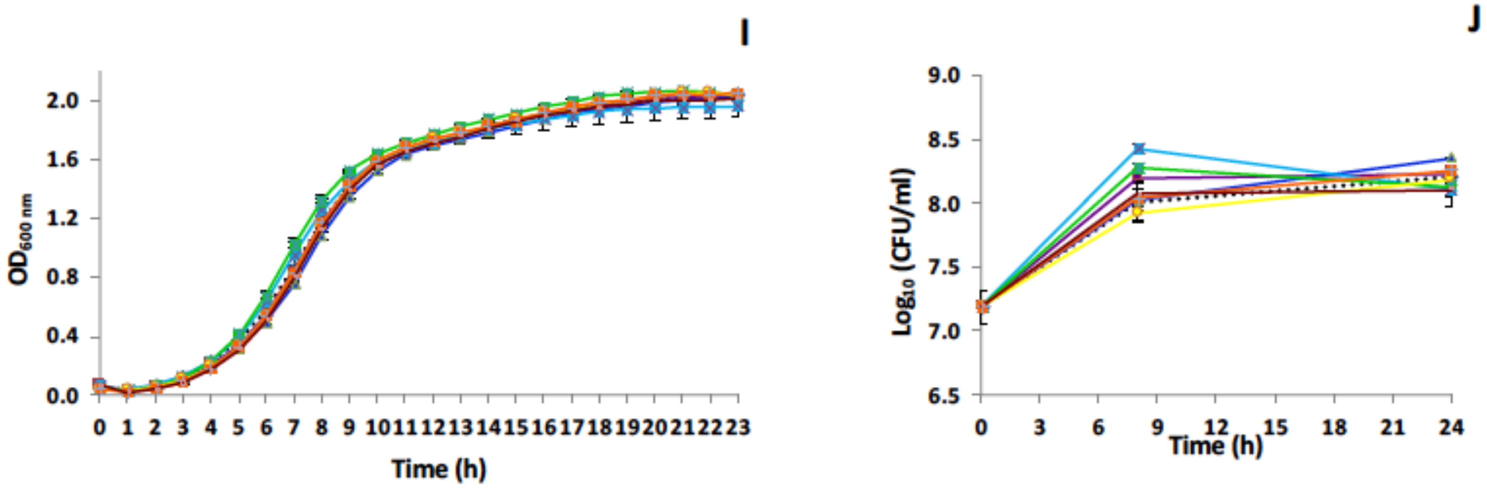
L. pentosus AP2-15



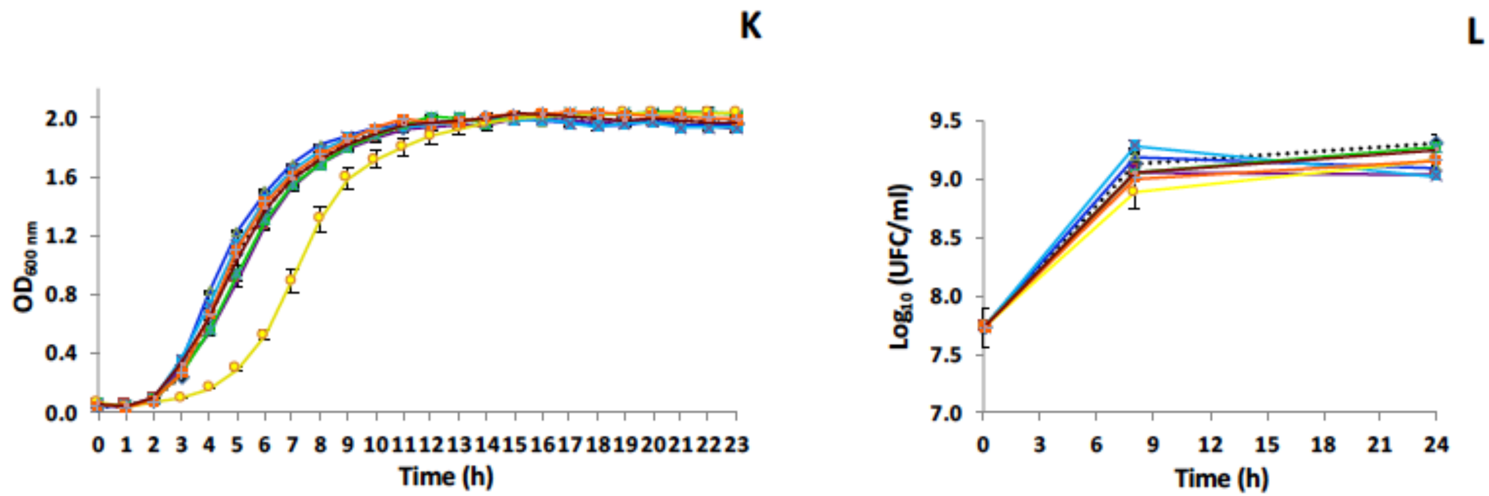
L. pentosus AP2-16



L. pentosus MP-10



L. pentosus CF2-10



L. pentosus CF1-39

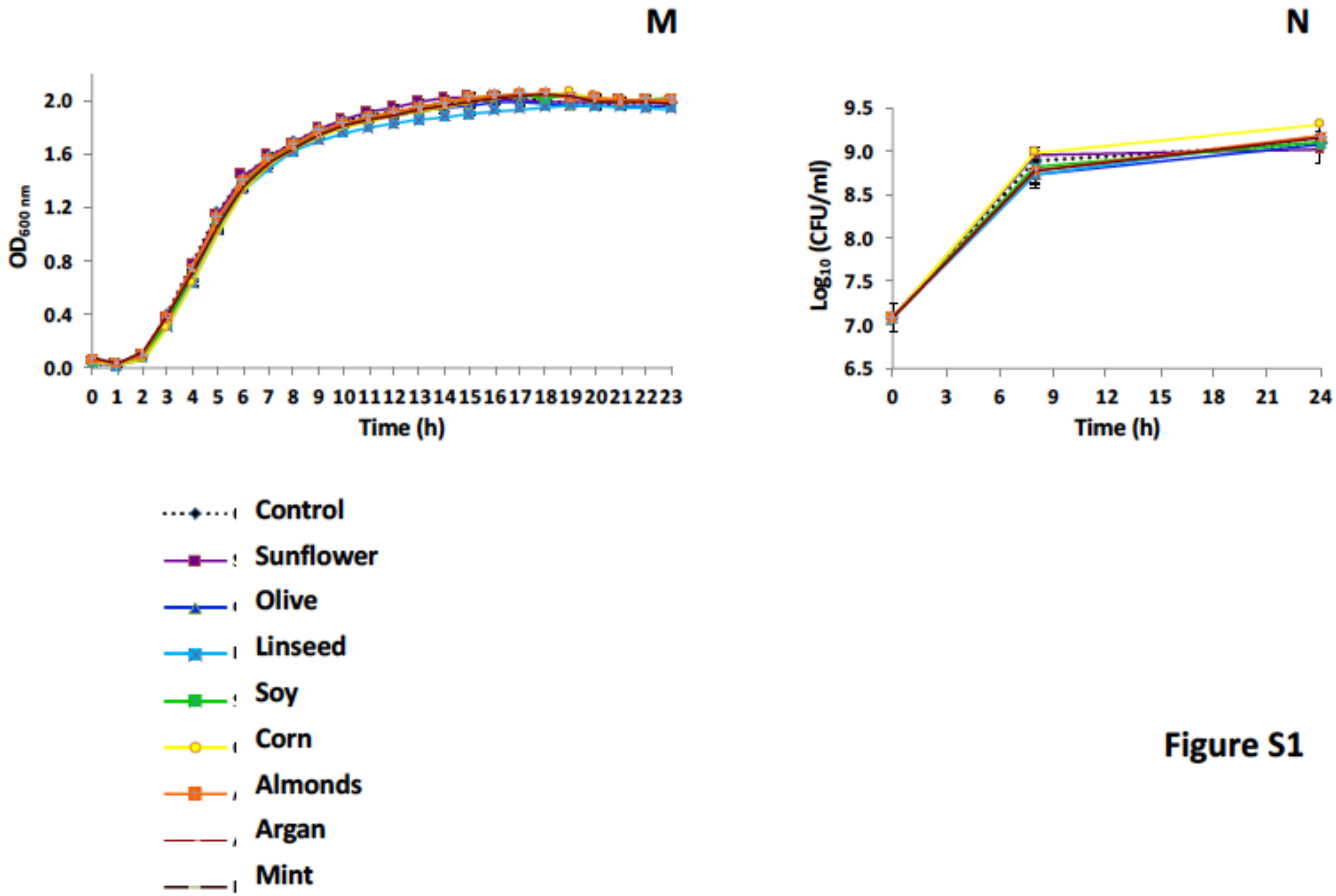


Figure S1